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JOHNE'S DISEASE

F. W. TWORT & G. L. INGRAM

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A MONOGRAPH ON JOHNE'S DISEASE

(ENTERITIS CHRONICA PSEUDOTUBERCULOSA BOVIS)

PLATE I.

SHORTHORN COW SUFFERING FROM JOHNE'S DISEASE.

Frontispiece.

A MONOGRAPH

ON

JOHNE'S DISEASE

(ENTERITIS CHRONICA PSEUDOTUBERCULOSA BOVIS)

BY

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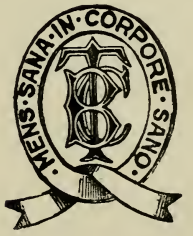
AND

G. L. Y. INGRAM, M.R.C.V.S.

LATE VETERINARY SURGEON TO THE BROWN INSTITUTION

ILLUSTRATED WITH NINE PLATES



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PREFACE

Johne's disease, the subject of this small monograph, is a condition that has recently attracted considerable attention in this country. In 1895 Johne and Frothingham discovered the causative bacillus in the lesions, and since that time the disease has been investigated by many other workers. Although at the present day Johne's disease is frequently mistaken for tuberculosis, strongylosis, and other conditions, it is rapidly becoming more generally recognized. It is known that besides cattle, sheep, deer, and goats may become infected, and that the disease is widely distributed over the globe, in some areas the loss to stockowners from this condition being greater than that from tuberculosis. An important article by Professor Penberthy dealing with the disease and its economic importance, and the need for State legislation, has recently appeared in the *Journal of the Bath and West of England Agricultural Society.*

In the following pages we have attempted to summarize existing knowledge on the subject, and have brought before English readers the investigations of Continental workers—notably those of Professor Bang of Copenhagen and of Professor Miessner of Hanover—which have not been generally available to veterinary surgeons and stockowners in this country. We have also included our own investigations, which have been

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carried out at the Brown Institution during 1910, 1911, 1912, and the first three months of the present year.

These investigations have thrown a considerable amount of work on the staff of the laboratories, and here we should like to record the industry and care with which the chief attendant, Mr. Frankham, has performed the various duties allotted to him.

The book consists of nine chapters, and throughout the descriptions and details refer to cattle unless otherwise specified. The first five chapters are allocated to the history of the disease, its importance to stockowners and breeders, its clinical features, methods of diagnosis and treatment, and the pathological lesions found post mortem.

In Chapter VI. we deal with the cultivation of Johne's bacillus, and describe in some detail the experiments that have been carried out in this direction by different workers from the time of Johne and Frothingham to the present day; we also include a description of the bacillus, and the appearance of the cultures on fluid and solid media.

In Chapter VII. we describe the method of preparing a specific diagnostic vaccine, and the earliest experiments carried out with avian and other tuberculins are also given. Chapter VIII. contains some results with agglutination and complement-fixation tests, which were performed by Dr. C. C. Twort. Chapter IX. contains most of the recorded experiments with infective material and pure cultures of the bacillus. Here, too, we include a large number of experiments by Dr. C. C. Twort and Mr. T. Craig on the small laboratory animals.

Research work on a disease which affects any of the larger domesticated animals is necessarily very costly, not only from the expense of the experimental subjects

but also from the cost of feeding and keeping them. On this account our experiments, though covering a fairly wide field, have not been so numerous in some cases as we should have wished. In view of the importance of this disease to agriculturists, the question is one which should be investigated with public money. In our work on this disease, however, we have received no assistance from the Board of Agriculture or from the Development Fund Commissioners, even though applications for a grant have been made after the essential part of our work—the cultivation of the bacillus—had been verified by the Danish Government veterinary bacteriologists. Under these circumstances, we wish to give especial notice to the generosity and kindness of many owners and breeders of cattle who have assisted our work with gifts of affected animals for experimental purposes, to the help afforded by a large number of veterinary surgeons who have sent specimens and answered questions on different points of interest, to the Royal Society for the monetary grants which made the earlier experiments possible, and to the University of London for the grants from the "Henry Dixon" Fund for research. We wish to thank Professor Dr. Miessner of Hanover, Dr. Halfdan Holth of Copenhagen, and Professor Penberthy for the loan of papers and for references to other authors, Monsieur Vukovic of Livno (Bosnia) for sending a paraffin block and details of his own work on the disease as it affects sheep, and Messrs. De Vine, Hamilton, Angwin Le Sueur, and other veterinary surgeons for kind assistance in many directions.

F. W. T.
G. L. Y. I.

April, 1913.

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JOHNE'S DISEASE

CHAPTER I

NOMENCLATURE, DEFINITION, HISTORY, AND DISTRIBUTION

Nomenclature—*English.*—Johne's disease. Chronic pseudo-tuberculous enteritis. For the condition in sheep, Scrapie or Scrapy (doubtful). Chronic bacterial dysentery (America).

French.—Entérite spézifique chronique des bœufs.

German.—Die spezifische chronische Enteritis des Rindes. Die chronische pseudotuberkulöse Darmentzündung des Rindes.

Danish.—Kvægets kroniske smitsomme Tarmbetændelse. Lollandske Syge.

Swiss.—Kaltbrändigkeit.

In Great Britain the disease to be described in this book is usually spoken of as "Johne's disease"—from the name of the discoverer of the acid-fast bacillus present in the lesions of cattle—as being less cumbrous than the more descriptive term, "chronic pseudo-tuberculous enteritis," which is commonly used in other countries. The latter name was suggested by Professor B. Bang in 1906, in which year he showed that the condition was a distinct disease in no way connected with tuberculosis; so that it has really more

claim to be called "Bang's disease," and its causal micro-organism "Johne's bacillus."

An objection to the term "pseudo-tuberculous enteritis" exists in the fact that there is never any resemblance between the macroscopic lesions of Johne's disease and those of tuberculosis, though under the microscope it is rarely possible to differentiate the causal micro-organisms. However, though for more than ten years after the discovery of acid-fast bacilli in the lesions the disease was regarded as a form of tuberculosis, and although the thickening of the bowel had been noticed by other observers previous to Johne and Frothingham, yet the name "Johne's disease" has been adopted in England, and nothing would be gained by attempting to alter it. In America it is known as "chronic bacterial dysentery," to which it may be objected that the passage of blood with the fæces is not a very common feature of the disease.

The popular terms for this disease are somewhat varied, and differ in different localities—"skinters," "scanters," "piners," and "wasters," are all terms applied to animals showing emaciation and diarrhœa. Possibly the term "waster" is more commonly applied to tubercular animals, in which the diarrhœa is less marked; but no reliance can be placed on the use of these terms by farmers.

"Scrapy" seems to be a term somewhat loosely applied to the disease when it affects sheep. Stockman mentions this term in his various articles on the subject, but states that it may be the irritable skin condition to which reference is made under this name, and which in sheep seems to be associated in some cases with bacterial enteritis.

"Lollandske Syge," or Laaland disease, is the old

name for the disease in Denmark, arising from the prevalence of the condition in cattle on that island.

"Kaltbrändigkeit," according to Meyer, is the common term for Johne's disease among Swiss farmers, in whose stock it occurs. The term merely describes the symptoms of "thirst without feverishness."

Definition.—A chronic specific enteritis, affecting cattle, more rarely sheep and deer, and probably goats and allied animals, caused by the multiplication, in the intestinal mucous membrane and mesenteric glands, of a specific micro-organism known as "Johne's bacillus," which produces a diffuse thickening of the bowel and an interference with food absorption, leading to diarrhœa and wasting.

History and Distribution.—The older writers on veterinary subjects devoted most of their attention to horses and horsemanship. From the time of Solleysel in France and Blundeville in England, both of whom wrote about the middle of the seventeenth century, the authors of numerous works on farriery that we have consulted merely give, as an appendix, a few prescriptions for common ailments of cattle. Skellet, in 1806, wrote a book on "Parturition in Cows and Diseases of Neat Cattle," in which he makes no reference to any condition comparable with Johne's disease. Some of these old writers, however, do mention that certain forms of diarrhœa in cattle are very chronic and incurable, and are "due to rottenness."

Hurtrel d'Arboval, who, in 1826, compiled, in French, a dictionary of the veterinary art, mentions, under the head of "Enteritis in Cattle," a thickening of the mucous membrane of the large and small intestines associated with chronic diarrhœa; but some of his

cases appear to have shown distinct ulceration, and were probably tubercular.

In the *Veterinarian* for 1831, Farrow and Cartwright both mention diarrhœa and wasting in cattle, and describe lesions which leave little doubt that the disease existed in England over eighty years ago.

B. Bang, of Copenhagen, states that, in 1881, Hansen, a veterinary surgeon of Nysted, in the island of Laaland, brought to his notice certain cattle in which chronic diarrhœa was accompanied by a thickening of the intestine. Later, Nielsen, on the same island, observed a similar condition on a large estate in his district. Bang made a post-mortem examination of a cow from this estate, but in this particular case the thickening was not very marked, and he was led to ascribe the diarrhœa to the irritation caused by small intestinal strongyles which were present.

In 1895, Johne, professor in the veterinary school at Dresden, and Frothingham, an American doctor working with him, first drew attention to the presence of acid-fast bacilli in the thickened intestine. Harms, a veterinary surgeon of Oldenburg, applied the tuberculin test to a six-year-old Oldenburg cow suffering from diarrhœa which he suggested was tubercular in origin. The injection of an ordinary dose of diagnostic tuberculin caused a rise of 1·6° C. in the temperature of the animal, the maximum reached being 39·6° C. As a result of this test the animal was slaughtered. Neither in the lungs nor in the lymphatic glands was any tubercular lesion demonstrable. The small intestine and cæcum were sent to Johne at Dresden. He found the ileum to be thickened in the manner now regarded as characteristic of Johne's disease, and on staining portions of the bowel with carbol-fuchsin, decolorizing with sulphuric acid, and counterstaining

—the method of staining now known as Ziehl-Neelsen's—he discovered the acid-fast micro-organisms which were then believed to be tubercle bacilli. The condition was therefore described as a hitherto unrecorded form of tubercular infection of the intestine. Cultures from parts of the intestine rich in bacilli were made on to glycerine-agar, but no growth was obtained. The inoculation of guinea-pigs with infective material also gave negative results. However, from the appearance of the bacilli in the lesions, the authors were led to attribute the condition to an infection with avian tubercle bacilli. Specimens were submitted to Robert Koch, who gave it as his opinion that the disease was either a true tuberculosis or was due to some modified or degenerate form of the tubercle bacillus.

The authors (Johne and Frothingham) concluded from their researches that the condition was a previously unrecognized form of tubercular enteritis due to an infection with the tubercle bacillus, or the avian tubercle bacillus, or a variation of one of these micro-organisms brought about by some change in nutrition, etc. Such a change, they thought, might be responsible for the alteration in virulence and for the negative or very slight reactions produced by the inoculation of ordinary diagnostic tuberculin into affected animals.

After Johne and Frothingham's original paper, the next important publication with regard to this disease came from Markus of Utrecht, in 1904. He stated that in Holland the disease had been recognized for many years by Koorevaar, who had repeatedly drawn attention to the thickened intestines of emaciated cattle showing no gross lesions in any other organ. He also stated that in some districts the condition in cattle

was known as "Scheisser," a name which is probably equivalent to the English term "scourer."

Markus himself found the causal bacilli in cases of Johne's disease, and introduced the *intra vitam* method of diagnosis that consists in taking scrapings from the rectal mucous membrane and staining films made from the material with carbol-fuchsin. He stated that at the abattoir in Amsterdam, van der Sluys had recognized eleven cases in one year. Markus made numerous cultural experiments which were entirely negative, and he inoculated rabbits, guinea-pigs, goats, and hens, with infective material without obtaining any positive result.

In 1905, Liénaux and van den Eeckhout, in the "Belgian Annals of Veterinary Medicine," gave an account of a study of the disease occurring as an enzootic in a herd of Jersey cattle, and also in native breeds in which they had encountered cases. After numerous experiments they concluded that they were dealing with a form of tuberculosis, being misled, in all probability, by the coexistence in some cases of tuberculosis and Johne's disease in the same animal. With experimental material from such animals tubercular lesions were produced in other animals by inoculation.

In the same year Borgeaud of Lausanne reported two cases—one in a five-year-old cow which was not markedly emaciated, and another in an animal which had suffered from periodic attacks of diarrhœa for about six months. In the latter case there was also tuberculosis of the bronchial glands and pleura. Inoculation of guinea-pigs with material from the infected gut produced abscesses in which there were acid-fast bacilli.

In 1906, Matthis of Lyons published an account of

the cases which he had met with in his neighbourhood; and in Germany, at the Berlin abattoir, Bongert investigated many cases. Both of these writers came to the conclusions arrived at by Johne and Frothingham, and considered the disease to be tubercular in nature.

In the same year B. Bang published an account of his inquiries into the disease in Denmark, and this authority must be credited with making the first real advance in our knowledge of this condition since the discovery of the acid-fast bacilli by Johne and his colleague. Bang first established the fact that the disease is a specific infection distinct from tuberculosis, and he suggested the name chronic pseudo-tuberculous (or paratuberculous) enteritis. He showed the existence of the disease among red Danish cattle and other native breeds, as well as in imported tubercle-free Jerseys.

By feeding two calves with the mucous membrane of the intestine of an infected animal, he was able to reproduce the disease. His attempts to cultivate the bacillus and produce lesions in guinea-pigs and rabbits were negative, and he decided that the condition was not a true tuberculosis. He also demonstrated its chronic nature and the long period of incubation. In the same year Bang gave an account of the disease before the National Veterinary Association at Liverpool, and showed specimens in illustration. He predicted that it would be recognized in this country, and mentioned that he had found it in tubercle-free Jersey cows imported from the Channel Islands. In the discussion that followed this paper, many of the practitioners present stated that they had been aware of the condition in their practices, but had not ascribed it to its true cause, intestinal strongyles usually being

considered as giving rise to the diarrhœa when probably Johne's disease was the cause.

In 1907 an account of six cases was given by M'Fadyean in the *Journal of Comparative Pathology.* These cases occurred in Shorthorn, Sussex, and Jersey cattle. An attempt to cultivate the bacillus was negative, and inoculation experiments also failed. Since this date very many cases have been recognized, and it is now generally admitted that the disease is prevalent all over England. Chase has seen it in imported Jerseys in South Africa; and Beebe, Pearson, and Melvin, have reported cases in North America. Throughout Germany the disease has been reported, and also in Schleswig-Holstein.

Meyer of Berne, in Switzerland, in 1908, gave a good description of Johne's disease—calling it "enteritis hypertrophica bovis specifica"—as it occurs in several of the Swiss cantons, and showed that it was in reality responsible for conditions commonly supposed to arise from strongylosis.

D. Meadows, of the Indian Civil Veterinary Department, has informed us that a colleague of his has observed a case in India, in Lahore, typical bacilli being present in smears made from rectal scrapings.

In England, Johne's disease is certainly very prevalent, much more so than is usually supposed. From inquiries made by owners of diseased cattle, and from veterinary surgeons, and also from replies to a circular letter sent to a large number of private practitioners and to those superintendents of municipal abattoirs who are veterinary surgeons, we have evidence of the existence of the disease in almost every county.

The condition is becoming much more frequently recognized and differentiated from other causes of

diarrhœa in cattle, but some considerable differences occur in the answers we have received. Generally speaking, the Midland Counties seem to be the worst affected. We have evidence of cases occurring in Devon and Cornwall, in Westmorland, and in Kent. Somerset appears to be badly affected, especially in the low-lying district round Bridgewater.

Edwards of Mold (Flintshire) states that on an average he meets with twenty cases a year. He writes: "I have not seen more than two cases on the same premises at the same time, but have had seven in the same place in one year. I think that a large number of cases are never attended by a veterinary surgeon; the farmers regard them as 'wasters,' and either attempt no treatment or use home remedies. . . ." In the North of Scotland cases are probably less common, though we have received specimens from Ross-shire and from the county of Lanark.

Sampson of Sheffield considers that the disease is on the increase in his district. Leicestershire in some parts is badly affected; so, too, is Northants, in which county one breeder has lost sixty beasts from this disease in the last seven years.

Perhaps the most accurate information can be gathered from a private communication from De Vine, who has taken a great interest in this condition. In the municipal abattoir at Birmingham about 25,000 head of cattle are slaughtered each year, or roughly 500 a week. Out of these 500 animals, about three cases of Johne's disease are noticed by De Vine in the ordinary course of meat inspection—that is to say, the animals are sufficiently affected to present macroscopic lesions. On making post-mortem examinations of animals killed from six to twelve months after inoculation with pure cultures, the present authors

have been able to recover the bacilli from the deeper layers of the mucous membrane of the intestine; but the thickening of the bowel in some of these cases was scarcely noticeable, and, indeed, infection could be proved only after a careful microscopic search, or by the cultivation of the bacillus. Yet, of those animals which on post-mortem examination show only slight lesions of Johne's disease, many are thin and in poor condition, being obviously affected by the bacilli or their products. It is reasonable, therefore, to suppose that in addition to the cases reported in the Birmingham abattoir, a good many occur which it is not possible to detect in the ordinary course of meat inspection. Besides these well-marked and slight cases, there must be added those animals which die of the disease, or are slaughtered at home as being useless to send to a properly inspected meat market, where, on account of extreme emaciation, they would be condemned. On the other hand, Parker of Newcastle, in answer to an inquiry, states that the condition is very rarely met with in the abattoirs in that city; and he accounts for this by the fact that only very good-class bullocks are slaughtered there.

In 1910, Riddoch, in inspecting about 2,800 dairy cows kept in the city of Edinburgh, diagnosed three cases by the examination of rectal scrapings; but he states that slaughterhouse statistics in the city would be of little value, as all the extreme cases would be totally condemned on account of emaciation, the cause of which would not be stated.

At Belfast the disease seems almost unknown, and at the Dublin abattoir distinctly uncommon. From County Armagh, Thompson writes that he is unaware of the existence of the disease in his district, and though the disease is met with in Limerick, Wallis

Hoare, of Cork, has not seen any cases in that district. From answers to other inquiries in Ireland, we suspect that many more cases occur than are recognized.

The Channel Islands are known to suffer heavy losses from Johne's disease, and are also remarkable for the scarcity of cases of tuberculosis in cattle. We have received most interesting information from Le Sueur of St. Heliers, who with Olaf Bang has investigated many cases of the disease.

He writes that he sees in his district, on the island of Jersey, about twenty-five cases a year, but rarely more than four on one farm. Only one breed of cattle is kept—Jersey—and the disease occurs most commonly in cows about two and a half years old, frequently after their first calf. He writes: "Johne's disease on the island chiefly affects young stock, and heifers after their first calving, and especially those reared on boggy land. Likewise among farmers who are milk-sellers, which means that they feed their calves, not on milk, but on some condiment, the animals being rather underfed. I also believe there is some tendency to hereditary transmission, having noticed many cases in the same family. I have also found that mature healthy animals (cows of five to six years old), when put on an infected farm, do not develop the disease."

The occurrence of the disease upon marshy or boggy land is generally recognized. Bang has found it prevalent in low-lying districts. Townsend finds it very common in the Fen districts of Lincolnshire, and many other observers (Le Sueur, Edwards, Scott) are agreed upon this point. Le Sueur writes: "The disease is unknown on some parts of the island—on high lands and under good farmers."

With regard to the prevalence of the disease in sheep

it is difficult to obtain reliable information. In 1907, Vukovic of Bosnia found cases of the disease in cattle, and in the following year he observed the same condition in sheep. We have received a communication from him with regard to the sheep, and he states that he has met with several badly contaminated flocks, although the majority of the flocks appear to be free from the disease. The cases mentioned by him were entirely in mountain sheep, and he makes the interesting observation that the disease seems to be most prevalent in leprous districts. Sections of the bowel of affected animals (made in 1909), sent to us by this authority, show acid-fast bacilli in enormous numbers.

Craik of Alnwick has brought cases of scrapie to the notice of the English Board of Agriculture, and Stockman has found acid-fast bacilli in the intestinal lesions similar to those of Johne's disease in cattle. From inquiries which we made by a circular letter to a large number of veterinary surgeons we have evidence of the existence of a similar condition in sheep with the clinical symptoms of Johne's disease, but usually the disease was not confirmed on post-mortem examination by the demonstration of the bacillus.

M'Fadyean, Sheather, and Edwards, have described a case in a Welsh ewe in which acid-fast bacilli were found in large numbers in the thickened wall of the intestine.

In the early part of this year the present writers received from Northampton a well-marked specimen of the disease in a sheep's gut, and it was from this specimen that we cultivated the sheep strain of Johne's bacillus mentioned later.

M'Fadyean, in his annual report to the Royal Agricultural Society, stated that he had met with a case in a herd of deer kept in a park, but, as far as we

have been able to ascertain, this is the only case recorded in this species of animal.

Quite recently a case has been reported in a horse by Liénaux; but this animal, which came from a farm, also showed definite evidence of tuberculosis in two of the abdominal glands, and as the lesions in the gut were evidently not typical, the case must be considered doubtful.

CHAPTER II

ECONOMIC IMPORTANCE AND ETIOLOGY

Economic Importance.—Until Johne's disease has for some time been scheduled under the Contagious Diseases of Animals Act, it will be difficult to estimate, with any degree of accuracy, the number of cattle affected with this disease. It may be granted that any animal which shows clinical symptoms is a source of loss to the owner; but an animal may become poor in condition and generally unthrifty from Johne's disease, though it may be killed before the onset of acute diarrhœa. The condition is not as yet generally recognized by stockowners and farmers. Among the smaller men especially, the "scanter" or "waster" is sold at a low price to a butcher or dealer, and in the latter case passed on to spread infection at the next market.

Harvey, of St. Columb, who has had many years' experience both of small farmers and of cattle breeding, points out the danger of the custom of selling a "scanter" with three or four sound bullocks, and suggests that the sale of an animal known to be affected should be made a punishable offence. This cannot be, however, until a test with a specific diagnostic vaccine has been recognized, and State legislation adopted.

In the milking breeds, Johne's disease is certainly

of the greatest importance, as it usually attacks cows, and causes death just at the time when they should be at their highest value—*i.e.*, after the second calf—when giving the best yield of milk. We have seen valuable three-year-old Jersey cows dying of this disease within a few weeks of calving, and a pedigree bull on the same farm reduced in value in the course of twelve months from over £100 to thirty shillings.

The loss sustained by an owner who attempts to treat the disease with extra food and drugs is fourfold; there is no return for the extra expense in feeding; there is less milk; a poor, weakly, or dead calf; and when in the end the animal is sent to the butcher a very low price is obtained. In the worst cases the animals are sent to the nearest kennels, or are buried on the farm. Besides these losses, there is the constant danger that healthy animals on the same land or in the same byre will become infected, and the loss is likely to become an annual one. The same may be said to hold good for tuberculosis in cattle; but while it is possible for a cow extensively affected with tuberculosis to be in fairly good condition, it is rare to find an animal badly affected with Johne's disease which is not so emaciated as to be wholly condemned in a properly inspected meat market.

A farmer who buys a good and recently calved dairy cow at the average price of £20, and only obtains 250 gallons of milk instead of 500 to 600, loses on the milk alone about £6. At the end of a year, in the case of a sound animal, he would expect to have a cow worth about £19, and a calf worth at least £1; but if the animal be badly affected with Johne's disease, he would be fortunate in obtaining £5, so that a loss of £20 is not an excessive estimate, as the milk has only been calculated at 6d. a gallon.

According to the returns of the Board of Agriculture for the year 1911, there are in Great Britain about 4,200,000 head of cattle over two years of age; of these 2,825,000 are cows in milk or in calf. If we take the low estimate of 1 per cent. of the milch cows alone as being affected with, and dying from, Johne's disease (or sold to a butcher when they should be at their best for milk purposes), and if we assume the loss on each to be that estimated above—*i.e.*, £20—then the annual loss in Great Britain would be over £500,000. But this takes no account of 1,250,000 beef animals, or of the extra value of pedigree animals, or of the possibility of many infected animals among the 26,000,000 sheep in Great Britain.

Bang quotes the figures in a circular issued by the Kustos Insurance Company, which insures a great number of cattle in Denmark (October, 1909). According to Bang, it is estimated that of 40 herds of Jersey cattle insured for about 1,000,000 kroner, 150 animals died in 29 herds. As compensation for deaths from this disease alone, the company paid out 28,000 kroner, which represents 3·5 per cent. of the total value for which the animals were insured. Presuming that the disease is equally prevalent among the 2,825,000 milch cows in Great Britain, and valuing them at £20 per head, the loss per annum involves over £1,000,000, and since it is unlikely that the disease is twice as prevalent in Denmark as in this country, our estimate of £500,000 is probably too low.

Etiology.—As was mentioned when dealing with the history of the disease, the association of a thickened intestine with symptoms of chronic diarrhœa and wasting had often been observed prior to Johne and Frothingham's discovery of acid-fast bacilli in the lesions. When B. Bang described the condition at

Liverpool in 1906, English veterinary surgeons at once recognized it as occurring in their experience.

Although Johne and Frothingham first observed the bacilli in 1895, they regarded them as tubercle bacilli in a modified form, and their opinion was upheld until 1906, when B. Bang proved the condition to be distinct from tuberculosis. He was able to reproduce the disease in calves by feeding them with large quantities of infected intestine; and Miessner and Trapp and Malm have confirmed his results.

In 1910 the present writers prepared a medium on which the bacillus was grown, and in the following year showed that the cultures fulfilled Koch's postulates; thus the undoubted relation of the bacillus to the disease was proved. For fuller details of this work, and a description of the morphology of the bacillus, the reader is referred to Chapter VI.

In the later stages of the disease the bacilli are discharged in large numbers in the fæces of the animal; and it is with these bacilli that the food supply and water become infected and the disease conveyed to other animals. There is now no doubt that the disease is not confined to cattle, but may attack sheep, deer, goats, and possibly allied animals. It occurs in animals of both sexes and all ages; though, from its chronicity, it is never observed in the very young. As has already been pointed out, a damp, marshy district seems to predispose to infection, possibly by allowing the specific bacillus to retain its vitality for a longer period outside the animal body.

The length of time the bacillus can live and remain infective outside the body has not been accurately determined, but it is probably very considerable. Although it is highly improbable that the bacillus can multiply in soil, it is quite conceivable that dung,

hauled in large quantities on to land that is used for growing swedes or other roots, is capable of infecting the roots, and so by this means the disease may be spread, as housed cattle often receive thirty to forty pounds of such roots daily.

Under natural conditions it is improbable that the majority of animals which ingest the specific bacillus would die of Johne's disease, even if kept alive for a sufficient time for the disease to develop. If this were so, then in a byre containing one badly infected animal among twenty healthy beasts, a much larger proportion would contract the disease than usually happens. According to statistics which we have been able to gather from veterinary surgeons, as a rule, not more than one or two cases occur on a farm in a year. The number of abortive infections must play an important part in determining the true infection of an animal, and possibly some strains of bacilli are more virulent than others, although inoculation experiments seem to show that the disease is not so readily contracted as tuberculosis.

Many authors are inclined to consider certain breeds of cattle, notably the Jersey, more susceptible than others to Johne's disease, while O. Bang states that the future of the Jersey breed in Denmark depends upon the possibility of eradicating pseudo-tuberculous enteritis from the herds.

M'Fadyean has recorded cases in Shorthorn, Sussex, and Jersey cattle; Angwin in Jerseys and Guernseys; Male in Jerseys and Devons. Chase has seen cases in South Africa which occurred in imported Jerseys; and one of the first cases to be reported in North America by L. Pearson occurred in a Jersey cow bred in that country. Townsend has seen cases in pedigree red-polled cattle, while M'Fadyean, Sheather, and Edwards

have recorded cases in Jerseys, Welsh, Shorthorn, and Devon cattle. The present writers have investigated cases in Shorthorns, Devons, Jerseys, and Herefords.

There seems to be but little doubt that in Denmark a greater percentage of Jersey cattle is affected than of the native breeds. This is shown by the Kustos Insurance Company in the figures relating to the compensation for losses due to Johne's disease in Jersey and Danish cattle. In 40 herds of Jersey cattle insured, the compensation paid was 28,000 kroner, and in 20 herds of native cattle, 6,000 kroner; from which it would appear that the disease is roughly twice as prevalent in Jersey cattle as in the native races.

Miessner and Trapp have made a very careful study of the condition, and consider it to be a stall disease (Stallseuche), or a disease occurring in animals kept housed for a great part of the year.

In a herd of Hereford cattle kept specially for beef purposes, the proportion of old cattle is very small when compared with the proportion of aged cows in a herd of Jerseys or milking Shorthorns. Where the sole or chief object is to produce milk, the animal is kept housed for a great part of the year, and especially is this the case in Denmark, where the system of dairy farming is more intensive than in England. It is also well known that farmers prefer to house their cows, with the idea that the greater warmth avoids the loss from the utilization of food for maintaining the animals' temperatures, and that by this means a better yield of milk is obtained. Then again, in a milk breed it is necessary for each cow to calve regularly, and the strain on the animal economy is very great. The well-known tendency of Johne's disease to appear suddenly soon after calving may be ascribed to the drain on the cow (she being already slightly affected

with Johne's disease) in providing for the needs of the growing fœtus, from the act of parturition, and from the sudden onset of the full activity of the mammary gland.

The possibility that one cow will infect a number of others may be greater when the animals are closely housed and are lying in pairs in stalls than when they are at pasture; for, as is well known, cattle usually avoid feeding on or near the masses of rank grass which grow where dung has been deposited. On the other hand, the infective material must be fairly generally distributed by rain, etc., so that the greater frequency of the disease in stall-fed animals is probably due more to a lowered resistance than to greater opportunities of infection.

On the whole, it is probable that the prevalence of the disease in a particular herd or race is determined rather by the condition of life under which the animals are kept and their feeding and general hygiene than by any inherited tendency towards infection. The cases seen by Le Sueur, occurring in succeeding generations of Jersey cows, are probably the result of the calves becoming infected from the fæces of the cows, and not of an infection *in utero* which is exceedingly unlikely to take place.

The frequency with which pedigree and "milk record" animals are sent about the country to shows, etc., and bought and sold or hired for breeding purposes makes it probable that there are very few districts free from the disease.

The etiological factors which determine the spread of the disease in other susceptible animals have not been accurately determined, but probably they are much the same as for cattle.

CHAPTER III

SYMPTOMS AND DIAGNOSIS

Clinical Symptoms.—In the early stages of Johne's disease there are no symptoms from which a positive diagnosis can be made. In inspecting a herd of cows in a byre at milking time, one or two may be picked out which are apparently unthrifty. Their coats are erect or staring, and their general condition is poorer than that of their neighbours, although they have received the same care and attention. Usually the history of these animals shows that the extra food given to improve their condition has produced no good effect. In course of time they have slight attacks of diarrhœa, lose more flesh, and give less milk. In the case of a pregnant cow, the additional strain of supplying the needs of the growing fœtus hastens the progress of the disease, the diarrhœa becomes more frequent and severe, and emaciation more marked. Parturition, and the consequent activity of the mammary gland, often produces a fatal result within a few months of calving. Sometimes the symptom of diarrhœa is not shown until after calving, and yet the cow may die within a few months of its onset. The course of the disease depends somewhat on the diet and general hygiene. Although Miessner considers it a disease of stall cattle (Stallseuche), as a rule the symptoms are increased in severity when the cattle

are turned out to pasture. In some cases it is possible to prolong the life of an affected animal for many months by placing it in a warm house and supplying an easily digestible nitrogenous diet. The diet seems to play an important part in the progress of the condition. We have produced the disease in several calves in inoculation experiments: six months after inoculation the animals were killed, and found to have lesions of the disease, and to show acid-fast bacilli in these lesions. When killed, the calves were in poor condition, thin and hide-bound. For some days one was unable to rise without assistance; but on post-mortem examination this animal showed tuberculosis of the mediastinal glands as well as Johne's disease. None of these calves, however, had shown signs of diarrhœa. From the time of inoculation onwards the fæces, examined frequently for the presence of acid-fast bacilli, gave negative results, although for about a fortnight the fæces of one calf contained a large number of semi-acid-fast bacilli. These calves were fed for a time on milk (previously boiled to avoid tubercular infection) with the addition of a little cod-liver oil; later they were given good meadow hay and occasionally a little lucerne or other green food.

Bang noticed diarrhœa in calves at the eighth month after feeding with large quantities of infected intestine, and probably, at grass, our own animals would have shown symptoms of diarrhœa; but in naturally infected animals, the period from the time the bacilli begin to multiply in the intestinal mucosa to the onset of diarrhœa is rarely less than six months, and in well-fed animals it may be much longer. Probably it will be shortened in the case of animals in exposed situations on poor pasture, in pregnant cows, and in those which are suffering from tuberculosis, strongylosis, or

actinomycosis of the tongue or maxillæ. The initial quantity of infective material taken in, and the frequency with which the infection is repeated, will, of course, play a considerable part in determining the severity of the symptoms and the time of their appearance.

In no case have we been able to ascertain that the act of rumination is suspended, or that there is a rise of temperature for any length of time. Most authors agree that the temperature rarely rises above normal, and that it is often subnormal, though in some cases Male has noticed a temperature of from 103° to 105° F. Angwin has rarely found it above 101° F. The possibility that fever, in cases of Johne's disease, may be due to some coexisting disease, such as tuberculosis, or to secondary infection with intestinal bacteria, must not be forgotten. In a naturally affected animal at the Brown Institution, kept under close observation for over twelve months, the temperature was almost always subnormal in the morning—about 99° F.—and rarely, if ever, rose above 103° F. Nor, in inoculated calves, has any important rise of temperature been noted that could not be attributed to some temporary digestive derangement or coexisting tuberculosis, etc.

Consequently, there are none of the classical symptoms of fever, and, unless the animal is also suffering from tuberculosis or pulmonary strongylosis (Hoose), there is no cough. The muzzle is moist, and the eyes are bright, though they may be sunken from the absence of intra-orbital fat. In advanced cases, the mucous membranes are pale and anæmic. The pulse is sometimes weak, and may be faster than normal.

As a rule the appetite is unimpaired, and the animal ruminates to the end. Even when very badly affected a cow will usually eat cake or corn, though hay may be

refused. Coarse, innutritious food, frozen roots, ensilage, etc., tend to aggravate the diarrhœa.

In spite of the absence of fever, there is usually great thirst, as a result of the watery condition of the fæces. This thirst, with no sign of fever, has given rise to the popular name "Kaltbrändigkeit," applied to the disease by Swiss farmers.

Angwin states that it is often difficult to get affected cows in calf; but the point is not of very great importance, as such animals should be slaughtered rather than bred from. He states also that he has never observed any œdema of the sternum or dewlap, or in the intermaxillary space, but admits that he has seen this point mentioned by lay writers. In this connection Le Sueur has given some useful information. With regard to the disease in the island of Jersey, he writes: ". . . the guiding symptoms which decide the farmer to destroy animals are œdematous swellings in the intermaxillary space and under the sternum, with, of course, diarrhœa, etc. . . ." We, too, have recently seen an œdema of the intermaxillary space in several instances.

The general symptoms are such that tuberculosis is usually suspected, and a negative result with ordinary tuberculin has almost come to be regarded as a positive proof of the existence of Johne's disease—other obvious conditions being eliminated.

Some observers, including Miessner, have stated that the fæces are frothy and full of air-bubbles. This is true is some cases, but cannot be considered as peculiar to the diarrhœa that arises from Johne's disease. The colour of the fæces is not, as a rule, markedly different from that of healthy animals on the same diet. Undigested particles of food can be detected, and when the diarrhœa is very acute,

shreds of blood-stained mucus may be seen in the dejecta.

The diarrhœa is often intermittent in character, and in some cases appears to recur at more or less regular intervals of about three or four weeks. Between the attacks the animal improves somewhat in condition. We have noticed that the bacilli may be very numerous in the fæces at the height of an attack of diarrhœa, and that as the dung becomes more normal in consistence, they are much less numerous. This point, however, has not been confirmed by the examination of a large number of cases.

In the more chronic cases, the attacks of diarrhœa seem to vary in intensity. Miessner and Trapp, who have made most careful records of cases of pseudo-tuberculous enteritis, tested the urine in several instances, but found no marked abnormalities. These authors also made blood counts in some cases, but found the normal cells in their usual numbers. They found the milk reduced in quantity; but after prolonged centrifuging at a high speed they were unable to find any bacilli in the sediment. Their inoculation experiments with centrifuged milk, urine, etc., are described in Chapter IX.

Symptoms in Sheep.—As has already been mentioned, Johne's disease in sheep has been described in Bosnia by Vukovic and in Great Britain by Stewart Stockman. More recently M'Fadyean, Sheather, and Edwards have reported a case in a Welsh ewe. In Scotland, where the first case of the disease in Great Britain was discovered in the investigation of an outbreak of a disease known locally as "Scrapy," the condition is said to be spreading. The following interesting note by Stockman appeared in the Report of the Board of Agriculture for 1909:

"*Scrapy.*—This is a disease of sheep which is only known in a limited area of Scotland; but it has been reported that the area is extending. On the latter point, however, the evidence available is not convincing, and it is difficult to get full information on account of the reticence observed by farmers. Apparently the disease only affects ewes after they have had two or three crops of lambs. The symptoms are rather peculiar, and may at first be confounded with scab. One of the first signs is an itchy condition of the skin, which gradually becomes worse, and compels the animals to constantly rub themselves against fixed objects. The skin, however, shows no eruption as in the case of scab, and no acari can be found. The affected animals become very much emaciated. Small abscesses which are probably only the indirect result of the disease may appear at various parts of the body, particularly about the head. Eventually the animals die from emaciation if they are not previously slaughtered; but affected animals may live on in a miserable condition for several months. Dipping has no curative effect; on the contrary, it seems to intensify the symptoms. If we accept the local statement that the disease is spreading, one is justified in thinking that this may be a disease which is transmissible either by direct or indirect infection. It has not been possible to obtain a great deal of material, and from local inquiry by one of the Board's Veterinary Inspectors it would appear that shepherds and farmers are inclined to class more than one disease with totally different symptoms under the head of Scrapy, although some of the ailing animals do not show the most prominent symptom of scratching, from

which the disease has received its local name. In the case of one animal which had been affected for several months a condition of the bowel somewhat resembling that present in Johne's disease was discovered at the post-mortem examination. Acid-fast bacilli, indistinguishable from those found in Johne's disease, were also present in large numbers under the microscope in a small area of the intestine. This important lesion, however, which seemed at first to throw an unexpected light upon the cause of the disease, has not again been discovered in a very restricted number of autopsies which it has been possible to make on other affected animals."

In another report on the subject this extract is quoted, and further details are given by the same author. He wrote:

"Since the above report was written further material has been received for which the writer has to thank Mr. Craik, M.R.C.V.S. The material in question was sent from a farm upon which the disease known as 'Scrapie' had not previously appeared; but the owner had, a year before, bought sixty-five sheep from another farm on which the disease had existed for several years. . . . Twelve animals out of sixty-five had died with similar symptoms to the one sent up for examination."

Mr. Craik's report stated that the animals had started to purge very severely during the last six weeks after they had been put on roots, and that the disease seemed to affect sheep in the same manner as Johne's disease affects cattle. As the result of further inquiry it was

stated that the sheep in question were not known to have suffered from itching of the skin, but the emaciation had been "continuous and prolonged."

The author concludes that the disease known as "Scrapie" is not necessarily a form of Johne's disease, as it is possible, of course, that two different diseases may affect the same animals. It is to be noted, moreover, that the three sheep examined, and mentioned in the extract from the annual report given above, showed the characteristic skin irritation; but in only one could the lesions of Johne's disease be found; while in the later outbreak classical symptoms of Johne's disease were observed, but no itching of the skin was noticed.

In the case examined by M'Fadyean, Sheather, and Edwards, the subject, a Welsh ewe, was suspected of suffering from parasitic gastro-enteritis, and on post-mortem examination many worms (*Strongylus cervicornis*) were found in the abomasum. The frequency with which sheep and lambs are attacked with parasitic gastro-enteritis, and the high mortality often observed, suggest, in such cases, the need for careful post-mortem examinations; as it is easy to overlook slight lesions of Johne's disease, especially in the presence of an apparently obvious cause, such as *Strongylus contortus*.

M'Gowan and Rettie have recently published an account of an examination of four sheep suffering from scrapie. They give as the usually recognized symptoms: "great emaciation, pruritus, wool rubbed off, ulcers on nose, top of head, and legs; bare callosities at root of tail and on either side of it; . . . rubbing against walls and fences, etc., bleaching of wool over back, but persistence of appetite. A secondary anæmia was present, and the temperature varied within normal limits." These authors found sarcosporidia in all four cases, and no acid-fast bacilli. A description of the

post-mortem examinations and a discussion of this infection is given on p. 37.

From the descriptions given by different authors, it is probable that many of the symptoms described under the heading of "Scrapy" and "Scrapie" are not caused by Johne's bacillus, but are due to entirely different causes. Indeed, it is unlikely that in an uncomplicated case of Johne's disease in a sheep the symptoms are very different from those observed in cattle. The skin lesions probably belong to another condition, and the name "Scrapy" should be reserved for this disease; while "Johne's Disease" should be the name given to the disease of sheep produced by an infection of Johne's bacillus in the gut and mesenteric glands. This view is now held by most workers.

The present writers observed no symptoms, except some wasting, in five sheep experimentally inoculated with pure cultures of Johne's bacillus isolated from a cow; but the animals were killed when the disease was in an early stage, and we know that in cattle the disease must be advanced before definite clinical symptoms become manifest.

In 1911 we inoculated two young goats with pure cultures of Johne's bacillus. Seven months later the animals were apparently in good health, except that one of them occasionally showed some slight diarrhœa. No rise of temperature had been noted, their appetites remained normal; yet on post-mortem examination both were found to be affected with Johne's disease, and showed the typical lesions (pp. 53 and 151).

In the case described by Liénaux in a horse, diarrhœa, emaciation, and fever were present; but as this animal was also suffering from tuberculosis, it is probable that the fever, at least, was caused by the tubercular lesions.

Differential Diagnosis (Clinical).—The causes that may give rise to diarrhœa in cattle are very varied, and include errors in feeding, frosted roots, mouldy corn or hay, irritants such as ricin or sand in feeding cakes, and various mineral poisons; also intestinal strongylosis, often associated in young stock with bronchial strongylosis; and, lastly, coccidiosis and tubercular enteritis.

The acute febrile conditions, such as anthrax, rinderpest, and malignant catarrh, may also give rise to diarrhœa, but need not here be considered.

In some parts of the country certain land seems to predispose to diarrhœa in cattle pastured on it. In Somerset such land is known as "teart" land or "scouring land." The exact reason for the effect that this land has on the animals is not known; but with such land the diarrhœa occurs when the animals are turned out, and ceases when they are removed. The disease also attacks a number of animals at the same time.

The irritants, which arise from unsound food, poisoning, etc., can be considered together. They are naturally the first causes to be sought for by a practitioner in conducting an inquiry on a farm. There will probably be several animals attacked at once. The symptoms as a rule are acute, and the diarrhœa is well marked before the animal has suffered sufficiently from toxic effects to become emaciated. It may be accompanied by fever, loss of appetite, and cessation of rumination; and an examination into the dietetic and hygienic management of the farm will soon reveal the cause.

There remain to be considered strongylosis, coccidiosis (psorospermosis), and tubercular enteritis. Miessner and Trapp have mentioned a case of sarcoma-

tosis of the bowel; the animal showed all the symptoms of pseudo-tuberculous enteritis, and the true cause of the symptoms was only discovered on post-mortem examination. This condition is probably sufficiently rare to be ignored from a practical point of view, though, from a pathological standpoint, the case is of interest.

Strongylosis.—Cattle and sheep, especially young stock, are frequently the hosts of various species of strongyles. These small parasites—worms, as they are commonly called—vary in length from 3 to 4 millimetres up to 20 to 30 millimetres, and they inhabit the abomasum, or fourth stomach, and the small intestine. There are numerous varieties, of which the commonest are:

In cattle ... *Strongylus convolutus*, *S. gracilis.*
In sheep ... *Strongylus cervicornis*, *S. contortus.*

The distinguishing features of these worms are their size, and the markings on their caudal appendages or bursæ. They affect sheep up to a year old, and sometimes adult animals; they also affect cattle up to two and a half years old. In adult animals their effects are very much less marked. The chief symptoms of their presence are diarrhœa, wasting, loss of appetite, fever, and thirst. As a rule, a number of animals are attacked at the same time, and in lambs the course of the disease is very rapid, and the mortality very high. In cattle the affection takes a more chronic course. The affected beasts shows signs of anæmia; their coats are rough and their skin inelastic (hidebound). There is a fœtid watery diarrhœa, and cases may be mistaken for tuberculosis or Johne's disease. M'Fadyean, Sheather, and Edwards have recorded a case of Johne's disease, in a Welsh ewe, in which

a considerable number of worms—*Strongylus cervicornis*—were found in the abomasum. Husk, or hoose (parasitic bronchitis), is frequently associated with intestinal strongylosis. Treatment is very often unsuccessful, and animals left untreated rarely recover. On post-mortem examination the mucous membrane of the stomach and intestines may be found to be ulcerated, and may show catarrhal inflammation. It is often slightly thickened, and of a dark livid colour; while a certain amount of gelatinous exudate is present. Very frequently the large intestine has the same appearance, and there may be some fluid in the peritoneal cavity.

The chief characteristics that distinguish this condition from Johne's disease are a high temperature—105° to 106° F.—and a more rapid course. In some cases animals, if severely affected, die in three to four days, and they rarely live more than a month. The post-mortem lesions are those of a catarrhal enteritis, and in the fæces or stomach contents the worms, embryos, or ova, can be found on microscopic examination. The best way to discover the parasites is to take a small quantity of the contents of the stomach, dilute well with water in a flat glass vessel, stir, and allow to settle. On decanting the water, and examining the sediment with a hand-glass, one can see the worms, or, if their appearance is familiar to the observer, they can be detected with the naked eye. To determine the species, the worms should be picked out with a needle for examination under a low power of the microscope. Seen with the naked eye, the parasites have the appearance of shreds of cotton, and are greyish white in colour, though in some instances they are brown or reddish from contained blood.

The discovery of these parasites in large numbers,

and the history of the case and of the farm on which it occurs, are, as a rule, sufficient to differentiate the two conditions. In the event of the discovery of only a few of these parasites, it is well to make film preparations from scrapings of the mucosa of the intestine, and to examine for acid-fast bacilli.

Treatment of such cases is usually best carried out by applications of lime or salt to the land and by proper drainage. It is also recommended that young sheep should not be allowed on land where old sheep have recently been kept.

Coccidiosis, Psorospermosis, Dysentère Rouge.—These terms are all applied to the disease of cattle that is caused by the *Coccidium* (*Eimeria*) *bovis*, discovered by Zürn in 1878. Cases have been reported in Great Britain by Gair (1898); also in Switzerland, France, Italy, Germany, East Africa, the Soudan, and the United States of America. When it attacks adult animals and assumes a chronic form, it may be mistaken for Johne's disease. We have seen this condition described under the latter name in an American agricultural journal. The chronic cases are, as a rule, sporadic, but in young animals it occurs as an enzootic.

The coccidium oocyst, the form in which it is most commonly met with in the fæces of cattle, is a round or oval body measuring from 14 to 16 μ, or in some cases from 20 to 30 μ ($\frac{1}{1500}$ to $\frac{1}{800}$ inch). This is smaller than the *Coccidium oviforme* of the rabbit, which is met with in many parts of Great Britain.

The cysts have "a highly resistant envelope, showing a doubly contoured outline when viewed through the microscope. In freshly voided fæces the contents of the oocyst in many instances entirely fill the shell,

through a rounding-up and collection of the cytoplasm into a granular ball-shaped mass lying within the doubly contoured cell of the oocyst. In such cases a clear space appears to intervene between the envelope of the cyst and its contents. A nucleus of fair size can usually be distinguished in fresh preparations." From this account of the coccidium, given by Jowett, who investigated cases at Capetown, it is obvious that an examination of the fæces of affected animals should allow a diagnosis to be made easily. Small pieces of mucus from the bowel may contain innumerable oocysts.

In Switzerland, where the condition is not rare, and where it has been studied by Hess and Guillebeau, it most frequently attacks animals at grass ; on a pasture or in a cowshed it may spread in rapid succession from one animal to another until the majority or all of the cattle become affected. It is most commonly met with in summer or autumn. The coccidia are supposed to be taken into the alimentary tract with the food and water, and by migrating into the epithelium they set up a severe inflammation (Friedberger and Fröhner). The lesions are those of acute enteritis ; the bowel contents may contain blood and the mucous membrane may be swollen, and in some places may show hæmorrhages.

Ostertag states that Maske commonly found coccidia, which caused tubercles about the size of a pin's head, in the fourth stomach and intestines of sheep. No mention is made of their causing symptoms, but more recently Martin has found coccidia in the intestines of young kids similar to those already described by Marotel under the name of *Coccidium Arloingi*. "The internal aspect of the intestine was strewn with a large number of small nodules or tumours . . . re-

sembling coccidian tumours in the intestine of a sheep." Some were deposited in the centres of the regions of Lieberkühn's glands, which were hypertrophied and dilated. The symptoms reported were "sudden onset of the disease, refusal of food, rapid emaciation and decubitus, and death in convulsions in three days." Hertzog states that the symptoms in sheep are similar to those in cattle, but that the coccidia are not found in the fæces. In the acute stages in cattle the presence of the coccidia in the fæces, and in sheep the discovery of the coccidia in the intestinal mucosa on post-mortem examination, should serve to differentiate this condition and Johne's disease.

Tubercular Enteritis.—According to Friedberger and Fröhner, from statistics of researches made throughout the German Empire, tuberculosis of the intestines is found in 1 per cent. of cattle suffering from tubercular disease.

Nocard, writing of tuberculosis in cattle, says:

> It is not rare to find miliary tubercles developed within the thickness of the mucosa, or in the submucous cellular tissue of the intestines. These tubercles may be isolated or agglomerated; in either case they quickly undergo softening, and empty their contents into the intestinal canal, thus causing a small wound of the mucosa, which has no tendency to heal; these ulcers, at first small and isolated, generally tend to spread. Such lesions are especially numerous in the last portions of the small intestine and in the cæcum. Perforation of the intestine is quite exceptional. . . . Intestinal and mesenteric tuberculosis causes frequent colic with profuse diarrhœa, alternating with obstinate constipation.

From this description—a translation of Nocard's article—it is evident that tubercular enteritis is not common. Examination of the fæces would, of course, be useless, as, if acid-fast bacilli were found, it would be impossible to decide whether they were Koch's or Johne's micro-organisms. An application of the tuberculin test should give a positive result, and as the disease is very unlikely to be cured or to show any improvement, immediate slaughter is advisable. The symptom of obstinate constipation, noted by Nocard, is important, as we have been unable to discover any record of such a complication in Johne's disease.

An interesting point has been raised by the discovery of M'Gowan and Rettie that four sheep suffering from scrapie and examined by them were heavily infected with *Sarcocystis tenella*. To a greater or less extent all the domesticated animals are from time to time found to be affected with sarcosporidiosis, and occasionally the parasites are sufficiently numerous to give rise to symptoms. Moule has found 98 per cent. of cachectic sheep and 34 per cent. of healthy sheep infected. He has also found the parasite in 46 per cent. of goats examined, and in 37 per cent. of oxen condemned for extreme emaciation. Besnoit and Robin, quoted by Gray, found a sarcocyst present in great numbers in a ten-year-old cow which showed marked skin lesions, thickening, depilations, etc. Though they were unable to inoculate the disease, they succeeded in causing death in a rabbit by injecting subcutaneously a glycerine extract of some of the larger lesions. Gray states that sarcocystin, consisting of a glycerine extract of the parasites, was found by Laveran and Mesnil, and also by Pfeiffer, to be toxic to rabbits, producing, when injected under the skin,

diarrhœa and paralysis, and causing death in seven to eight hours.

M'Gowan and Rettie, in the four cases of scrapie mentioned, found, post mortem, no naked-eye lesions except strongylosis of the lung and alimentary canal. The extent of this invasion of strongyles is not mentioned. The lesion common to all the animals was a marked sarcosporidiosis, which was only seen on microscopic examination. The parasites were found in the panniculus carnosus, in the muscles of the jaws, pharynx, larynx, neck, pelvis, and hind limbs; also in the intercostals, heart, œsophagus, and diaphragm. Though carefully sought for, no acid-fast bacilli were found in the intestinal canal or in the mesenteric glands.

In swine the sarcocyst infection is said to cause paralysis of the hindquarters, stiffness, and cutaneous eruption; but none of these symptoms, mentioned by various writers, can be said to be characteristic, as they may be met with in so many other diseases of swine.

However, it may be shown by future investigations that the skin lesions and pruritus are due to a sarcocyst infection, and that diarrhœa and emaciation are the only symptoms caused by the acid-fast bacilli in the intestines of sheep, as in cattle. The bacterial infection of the bowel may assist the sarcocysts in becoming dangerously numerous, and these parasites may lower the power of resistance in the sheep so that the bacilli can establish themselves. M'Gowan and Rettie's suggestion as to the pruritus, etc., being caused by the sarcocysts bears out Stockman's theory that the enteritis and the skin lesions in these cases are distinct.

Diagnosis (Bacteriological).—Since the recognition of Johne's disease is becoming more general in Great

Britain, the question of a certain means of diagnosis is now of great importance. Recently the case of a warranted cow developing Johne's disease within a month of sale has been the subject of legal comment in an agricultural newspaper.

As a rule the attention of a practitioner is called to a cow in poor condition which is suffering from diarrhœa, and there may be a history of other animals having been similarly affected during the past year. If he applies the ordinary tuberculin test with a negative result, the suspicion of Johne's disease is certainly increased, and the fæces should then be examined for acid-fast bacilli.

Meyer's procedure is probably the best. A small quantity of liquid fæces should be placed on a flat dish, and in this thin layer it may be possible to see small shreds of mucus. The blade of a small knife may then be passed through the flame of a spirit-lamp and a piece of mucus picked out and rubbed over the centre of an ordinary glass microscope slide. The film is dried and stained by Ziehl-Neelsen's method (p. 64). The presence in such a film of acid-fast bacilli in every way resembling Johne's bacillus is strong evidence that the animal in question is suffering from Johne's disease; but there are acid-fast bacilli, such as dung bacilli (mist bacillus of Moeller) and the timothy-grass bacillus, which may easily be mistaken for the bacillus of pseudo-tuberculous enteritis.

A better method is to take scrapings from the rectal mucous membrane or to pinch off a small portion of this with the finger-nails by inserting the arm into the rectum, since the bacilli so found are more likely to be the specific infecting micro-organism. The latter procedure, however, is open to the objection that although films made from the walls of the ileum and cæcum and

part of the large intestine may show enormous numbers of bacilli, yet the disease may not extend to such parts of the bowel as are within reach of an arm or of a curette. This is well illustrated in the graphic records of the comparative numbers of bacilli in scrapings taken from the mucosa of each 10 feet of the intestines of cattle killed while suffering from Johne's disease, drawn up by M'Fadyean, Sheather, and Edwards. These authors' results show that by far the largest number of acid-fast bacilli are found in the neighbourhood of the ileo-cæcal valve, and that the number falls off almost to nil after the rectum is reached, though in some cases films from rectal scrapings are very rich in acid-fast bacilli. This agrees with the experience of the present writers.

For these reasons the examination of scrapings from the rectum cannot be relied upon, and a negative result is valueless. Reichel and Deubler, as the result of an examination for tubercle bacilli in the fæces of bovines suffering from tuberculosis, arrived at the following conclusion: Microscopical examination of fæces and rectal scrapings is of no value, on account of the frequent presence of bacilli with the morphological and staining characters of the tubercle bacillus, but which are not tubercle bacilli.

The problem of obtaining a reliable method of diagnosis was nearer solution when O. Bang, in 1909, found that a certain proportion of cattle suffering from Johne's disease reacted to avian tuberculin, in the same way that tubercular cattle react to ordinary tuberculin. In the absence of an autogenous vaccine, which it was impossible to obtain until the specific bacillus had been cultivated outside the animal body, O. Bang's reagent was the best so far obtained; but in the hands of English users, and in our own experience,

it is not sufficiently reliable to be used with reasonable safety, as the large dose which has to be given (8 to 10 c.c. in adult bovines) is likely to give a reaction with other conditions (see discussion under Vaccines, Chapter VII.).

The present writers have obtained marked rises in the temperatures of naturally affected animals and of inoculated calves, goats, and sheep, by using a diagnostic vaccine prepared from pure cultures of Johne's bacillus. The cultures were grown on special media and also on ordinary broth after acclimatization of the bacillus. Holth also has obtained a typical reaction, with an autogenous vaccine, in a calf which had been inoculated three months previously with a pure culture of Johne's bacillus, although in this instance an extract of the tubercle bacillus was also present in the fluid. In the opinion of the writers, a vaccine prepared from cultures of the specific bacillus is the only reliable diagnostic reagent for Johne's disease, and in all doubtful cases tests with such a vaccine should be carried out. For full details the reader must refer to Chapter VII. It only remains to be noted here that very advanced cases—which, however, can be diagnosed quite easily from the clinical symptoms, etc.—may fail to react, a result which is also frequently obtained when advanced cases of tuberculosis are tested with tuberculin. Our experiments indicate that the autogenous vaccine for Johne's disease is quite as reliable as tuberculin is for tuberculosis.

CHAPTER IV

PROGNOSIS, TREATMENT, AND PROPHYLAXIS

Prognosis.—It has been stated that in some cases of Johne's disease recovery takes place. Le Sueur has informed us that in Jersey he has noted that adult cows put on to affected land do not contract the disease. If these animals have not been previously in contact with affected cattle or exposed to risk of infection, it must be supposed that the natural resistance to the disease is increased with age; but it may be that the animals, having already contracted the disease in a mild form, and having subsequently recovered before the development of clinical symptoms, have acquired thereby an active immunity.

M'Fadyean mentions a case that he believed to be one of recovery. He says: "The exceptional case was a yearling which, along with a cow on the same farm, developed symptoms of the disease. . . . Soon afterwards the cow was killed and the disease verified at the post-mortem examination. The yearling, although not medicinally treated, gradually ceased to scour, and gained a little in condition. It was killed six months after admission, and the post-mortem examination showed that the intestine was normal in appearance, while none of the characteristic bacilli could be detected with the microscope." In the case thus described the animal had certainly been exposed to great

risk of infection; but as, apparently, no acid-fast bacilli were at any time found in the fæces, and none in the bowel wall at the post-mortem examination, it is possible that the original diarrhœa was due to some other cause, especially as the animal was a young one.

B. Bang mentions that he has noticed that cows, after showing symptoms of the disease (such as diarrhœa, slight wasting, etc.), and then apparently recovering for a while, die in the end of Johne's disease. We have been unable to find any record of a case, where the bacilli have been detected in the fæces, in which the animal has been known to recover and to remain in normal health for any considerable time afterwards. It is highly probable that once the bacilli multiply to any extent in the intestinal mucosa the animal will die of the disease after a period of from three months to two years, depending on the sex of the animal, its food supply and hygienic management, and on the presence or absence of other diseases, such as tuberculosis, etc.

It may be assumed, therefore, that a prognosis of recovery is not possible in any clinically affected case. The possibility of treatment with a curative vaccine, when the disease is discovered in a very early stage by means of a diagnostic reagent, can only be determined after a considerable number of experiments. Nevertheless, our knowledge of allied diseases such as tuberculosis leads us to believe that such treatment might lead to recovery.

Treatment.—Unfortunately, there is no specific treatment for Johne's disease. We know of no drug that will kill the bacillus in the pathological lesions or neutralize the toxins which it produces. Moreover, the risk that one diseased animal will infect others on the farm is so great that by far the safest, and in the end the cheapest, course is to kill all the infected

animals—not only those which show clinical symptoms, but also any that may react to a dose of diagnostic vaccine prepared from cultures of Johne's bacillus. As to whether an attempt should be made to improve the condition of an affected animal before it is sent to the butcher, the circumstances of the case must decide. Should, however, an attempt be made to cure an affected animal, complete isolation should be insisted on; the dung, litter, etc., should be burnt, and a separate pail kept for milking, if necessary.

All the drugs that are usually employed as intestinal astringents, as tonics, and as stimulants seem to have been tried in Johne's disease, but in no case with success. Miessner and Trapp record that tannoform, creolin, starch gruels, iron, and various tonics, have all given negative results in the hands of practitioners who have brought cases of the disease to their notice in the north-west of Germany.

In England, dilute sulphuric acid, tobacco in the form of balls, nitro-hydrochloric acid, cyllin, solutions of copper sulphate, perchloride of iron, and other similar reagents, combined with such foods and gruels as are usually employed in cases of diarrhœa, have been given without success. Astringents and tonics (combined with good, easily digestible nitrogenous food, with small quantities of roots or green food, and a restricted allowance of water) may possibly be used in some cases with temporary advantage, but there is no certainty in the results.

Curative vaccines, analogous to the curative tuberculins used in the human subject, can, of course, be prepared. Indeed, in testing a badly affected animal with various weak diagnostic vaccines, we have noticed some considerable improvement in condition after the administration of these reagents, and it is

only lack of opportunity from financial reasons which has prevented our experimenting on these lines. If the disease were detected in an early stage with the diagnostic vaccine, such treatment might be both useful and of practical value in the case of pedigree bulls.

Prophylaxis.—In the present state of our knowledge the question of prophylaxis is of much greater importance than that of treatment. As soon as the diseased animals are removed from the farm, all buildings, etc., in which they have been housed should be effectively disinfected. Where possible, the floor of the cowshed should be scraped, and if the floor is an earthen one, the scrapings should be burnt. Cement floors should be washed with hot solutions of some reliable disinfectant, and probably roughening the old floor and the application of a fresh layer of cement would be a good procedure, if combined with several white-washings of the rest of the building and its fixtures with lime mixed with carbolic acid, etc. All dung, litter, stale fodder, etc., from the neighbourhood of affected animals should be burnt or ploughed in on land used for root crops, though the latter is not free from all risk. Scrapers, milking-stools, milkers' coats and aprons, spans, neck chains (or ropes), buckets, and any other utensils in the byre or milking shed should be treated with disinfectants or thrown away, and new articles substituted.

When affected animals have been isolated in a particular field, or where a field is known to be badly contaminated, it might be advisable to plough it up and bare fallow it during the summer, and then use it for growing some crop that will not be given to cattle or sheep. It is improbable that the disease can affect pigs, and as only one doubtful case has been recorded in the horse, these animals are not likely to contract

infection; cattle or sheep, however, should be kept off land known to be infected.

Sheep must be considered as carriers of the infection; and in carrying out prophylactic measures on a farm these animals should be inspected. Weakly and emaciated members of the flock should be carefully isolated, and if showing signs of diarrhœa, the fæces should be examined microscopically. Such animals should also be tested with a diagnostic vaccine prepared from Johne's bacillus.

In buying new stock, whether sheep or cattle, their history should, if possible, be known, and cattle should certainly be tested before being allowed to run with other animals on the farm. In the case of cows known to have come from an affected farm, it might be arranged in a warranty that the purchases have passed a test with a specific vaccine shortly before the sale. To be really effectual, all these prophylactic measures should be carried out under the personal supervision of a veterinary surgeon, and the best means of eradicating the disease is to test the whole herd every three months in order to get rid of reacting animals before they commence to excrete the causal bacilli in their fæces in large numbers, and so expose their neighbours to the risk of infection.

CHAPTER V

POST-MORTEM EXAMINATION, PATHOLOGICAL ANATOMY, AND PATHOLOGICAL HISTOLOGY

Post-mortem Examination and Pathological Anatomy.—In making a post-mortem examination of a case of Johne's disease one should follow the ordinary procedure. It is well in the case of a cow to examine the supramammary lymphatic glands for evidence of tuberculosis. The abdominal cavity should be opened, and the stomachs and intestines, together with their lymphatic glands, must be removed for a closer scrutiny. The remaining abdominal organs, the thorax with its contents and glands, and the pharyngeal lymphatic glands, should also be inspected for evidence of tuberculosis and other pathological conditions. The stomachs having been opened, they should be examined for any abnormalities; this applies particularly to the abomasum, in which strongyles may be found.

The intestines must be slit up their entire length with bowel scissors, or with a sharp knife with a cork on its point, and lightly washed to rid them of ingesta, a large sink being convenient for this purpose.

Perhaps the most striking feature of this disease is the insignificant character of the lesions met with in comparison with the state of emaciation to which affected animals are, as a rule, reduced, and the fact

that, beyond the intestinal mucosa and the colic and mesenteric lymphatic glands, no other lesions containing bacilli in a naturally contracted case have been recorded. As a rule the ileo-cæcal valve and the last part of the ileum may be expected to show the most marked lesions.

Even when experimental animals are fed with infective material, or pure cultures of Johne's bacillus, or are inoculated intravenously, intraperitoneally, or subcutaneously, the disease is developed in the small intestine; and except in the case of subcutaneous injections, which in some cases have caused a small localized abscess, no other lesions containing bacilli are demonstrable post mortem.

Before cutting into the intestine one can often perceive the thickening of the small bowel by taking it between the finger and thumb and comparing it in various places. In a bad case some parts are appreciably thicker than others, but there is never any evidence of the disease on the peritoneal membranes. A variable length of the intestinal tract seems to be affected. Measured from the ileo-cæcal valve, the small intestine for about 30 feet often shows evidence of disease; sometimes the length infected is as much as 70 feet. The initial part of the duodenum in cattle seems never to be affected; but the disease may extend as high up as Vater's ampulla, and in one of our naturally infected cases this was extensively involved.

A badly-affected piece of bowel shows marked thickening, and the mucous membrane is thrown into characteristic corrugations. These corrugations are often broken up into small islands which give a warty appearance to the surface of the gut. In a piece of normal small intestine from which the fat, etc., in the

mesentery has been cleared away, and which has been cut open longitudinally, a number of small folds are seen; but if the bowel is stretched as widely as possible these folds disappear. In a case of Johne's disease it is not possible to make these corrugations assume the level of the normal intestine. The same remarks apply to the cæcum, which is very frequently affected, though in this part of the bowel the lesions are inclined to be more patchy.

Occasionally the thickening is limited to small portions of the bowel; a few feet may be markedly affected, then an interval of sound or apparently sound intestine may intervene, and then another portion of diseased intestine may be found. The small intestine may escape entirely, and lesions be present only in parts of the large bowel. Apparently the naked-eye evidence of the disease is no guide to the number of bacilli that may be found beneath the surface. This has been well shown in a case examined by M'Fadyean, Sheather, and Edwards, in which, post mortem, the small intestine showed no macroscopic evidence of disease; yet smears taken from the mucosa of the last 90 feet were found to be quite rich in acid-fast bacilli.

If a portion of intestine is badly affected acid-fast bacilli are, as a rule, to be found in the neighbouring lymphatic glands; but this is subject to considerable variation, as in some cases the bacilli are unexpectedly numerous, and in others none can be found. The colour of the corrugated mucous membrane in a well-marked case is rather characteristic, being of a pinkish-yellow tint, with occasional patches of congestion; but there is never any erosion of the surface of the mucosa, or any tendency to ulceration, unless the animal has been killed shortly after a dose of diagnostic vaccine

PLATE II.

[*Photo by F. Holmes, Bristol.*

LOWER PART OF ILEUM FROM A COW AFFECTED WITH JOHNE'S DISEASE.

[*To face page* 48.

has been given, when we have found that hæmorrhages, and even superficial ulceration, may occur. In some cases, however, the mucosa is quite pale and anæmic. The rectum frequently shows transverse markings, and is often congested. The lymphatic glands are usually enlarged; but are rarely so to any extent, as may be the case in tuberculosis. They are more or less pigmented, and when cut into the pigmentation is found to be especially marked in the medulla. An œdematous condition of the glands is present, and on section a watery fluid exudes.

From the above it will be clear that, when well marked, the lesions are easily appreciable to the naked eye; but on post-mortem examination of a suspected case frequent scrapings should be taken throughout the length of the ileum and large intestine. In several cases the authors have been able to isolate the bacillus in pure culture when apparently there were no lesions, and when half an hour's search was necessary to find an area showing any bacilli; so that in a doubtful case cultures should be made on the special media. The ileo-cæcal valve and *adjacent* lymphatic glands should be examined most carefully, as these are the situations in which the disease is most frequently found in very early cases.

In some naturally infected animals we have observed a fatty condition of the liver, a condition that may also be found in cases of tuberculosis.

In the post-mortem appearances of sheep there appears to be nothing to distinguish the lesions from those occurring in cattle. The condition is of sufficient interest to warrant the quotation, *in extenso*, of the descriptions of the cases investigated by Stockman, and by M'Fadyean, Sheather, and Edwards.

The sheep forwarded to the Board of Agricul-

ture for post-mortem examination are described as follows:

"The lungs and heart were normal; the liver was fatty; the kidneys were normal, the rumen, reticulum, and the omasum were also normal; but the abomasum showed a few small areas of congestion. The small intestine was thickened, and showed numerous small hæmorrhages under the mucous membrane. The surface of the mucous membrane was coated with a white creamy mucus, but it showed no corrugations similar to those found in Johne's disease in cattle. The large intestine showed zebra markings and a few small hæmorrhages under the mucous coat.

"*Microscopical Examination.*—Scrapings from the mucous surface of the small intestine showed enormous numbers of acid-fast bacilli, mostly arranged in dense clumps, and indistinguishable from those found in Johne's disease of cattle. Scrapings from the large intestine also showed a considerable number of the same bacilli, but the microbes were much fewer than in preparations made from the small intestine. The same bacilli were also found in considerable numbers in smears made from the mesenteric lymphatic glands. Sections of the small intestines, made after embedding in paraffin, showed a colossal number of acid-fast bacilli. These were present as single elements and as dense clumps. They were most numerous towards the surface of the mucous membrane, but were also present in considerable numbers in the deepest part of the glandular layer. Microscopical sections of the mesenteric glands also showed numbers of bacilli, and nothing resembling a tuberculous formation could be seen.

"Inoculation experiments performed on the small animals of the laboratory showed that the acid-fast bacilli were not those of tuberculosis."

M'Fadyean, Sheather, and Edwards, give the following details of a post-mortem examination of a Welsh ewe, examined in April, 1912:

"A considerable number of worms (*Strongylus cervicornis*) were found in the abomasum, which was nearly empty, but no abnormal appearance of the mucous membrane was observed. The large and small intestines were slit up along their entire length and searched for worm parasites. One tapeworm was found.

"There was distinct thickening of the wall in the case of the large intestine, this being most marked in the cæcum; there was also some irregular wrinkling in this position. The thickening, although not so pronounced, was continued throughout the large intestine. The posterior half of the small intestine was appreciably thicker than normal, and at some places there was abnormal permanent wrinkling, though not so marked as is usually the case in cattle.

"*Microscopic Examination of Smears.* — This showed a rich invasion of the intestinal mucous membrane with small acid-fast bacilli quite indistinguishable from those usually found in Johne's disease of cattle. . . . The smear taken from the cæcum proved to be extraordinarily rich."

Bacilli were found in parts of the small intestine, but only a few in the colon.

In two sheep, which the present writers inoculated with pure cultures of Johne's bacillus isolated from a cow, and which were killed ten weeks later—after

obtaining positive reactions with a specific diagnostic vaccine—the following condition was observed post mortem: The animals were somewhat wasted, and showed enlarged glands throughout the abdominal cavity and thorax. When opened, the glands showed an œdematous condition, and some pigmentation most marked in the medulla.

In the animal inoculated intravenously the intestines were distinctly thickened, especially in the vicinity of the ileo-cæcal valve; but in the animal inoculated intraperitoneally there was no evidence of thickening.

Both cases showed only a few bacilli in the lesions, and none were found in the thoracic glands.

The condition of these sheep was identical with that observed in the experimentally inoculated calves which were killed before the disease had become advanced.

The liver in the sheep appeared to be fatty, and presented the same appearance as that sometimes met with in cattle.

M'Fadyean, in his Annual Report of the Royal Veterinary College, published in the *Journal of the Royal Agricultural Society*, 1907, wrote:

> ". . . . In ascertaining the cause of chronic diarrhœa among deer kept in a park, and when one of the diseased animals was killed the post-mortem examination unexpectedly disclosed the fact that it was the subject of Johne's disease. The fact that deer may suffer from the disease is not one of much direct interest, but it is of importance as suggesting that other ruminants, such as the sheep, may not be immune."

In July, 1911, the present authors inoculated two goats with pure cultures of Johne's bacillus. Goat 1 received intravenously 3 c.c. of an emulsion of bacilli

in sterile 0·85 per cent. sodium chloride. Twelve months later the animal was killed, and showed typical and well-marked lesions of Johne's disease throughout the intestines. The mesenteric glands were enlarged and œdematous. The bacilli were present in the lesions in fair number. Goat 2, which had received 1 c.c. intraperitoneally, showed the disease in a very early stage, and only very few bacilli were found. The lesions present in these animals showed the same characters as those already described in the case of bovines, and, in spite of the methods of inoculation, were confined to the intestine and neighbouring glands. The bacillus was afterwards recovered from these cases in pure culture (p. 152).

Liénaux gives the following account of the lesions found in the horse which he recently described. The account is taken from the translation which appeared in the *Veterinary Record* of April 19, 1913:

> "Post mortem, two lymphatic glands—one in the great mesentery and one in the meso-colon—were found absolutely caseous, though little enlarged. These were the only lesions of tuberculosis present. But extensive and serious lesions of the intestine existed, which were especially well marked upon the cæcum and double colon. The walls of these two viscera were thicker and more consistent than normal, and the small intestine was altered in the same direction, though to a much less degree. The lymphatic glands disposed along the cæcal and colic arteries were slightly tumefied and turgescent, and along the same vessels large distended lymphatic trunks, filled with yellowish lymph, could be distinguished.
>
> "The mucous membranes of the cæcum and large colon were very thick. Their free surface

was irregular, and was strewn with projections reaching up to 2 millimetres ($\frac{1}{12}$ inch) in height, and of very variable surface dimensions. Sometimes these projections took the form of well-defined nodules of hemp-seed size, in other cases —and these were more frequent—they took the form of more or less extensive raised plates, between which the mucous membrane appeared smooth and comparatively normal in aspect. Nodules and plates were in close proximity to each other, so that the depressed areas between them generally formed narrow folds. The surfaces of these nodules and plates showed losses of substance, which were punctiform upon the nodules but larger upon the plates, where it was impossible not to recognize their ulcerous nature. These ulcers were always shallow, and were nearly always covered with vegetable débris, which adhered to them rather closely.

"Microscopical examination of scrapings from the lymphatic glands involved, stained by Ziehl's method, showed bacilli isolated or in clusters, as they are found in the mesenteric glands of cattle affected with Johne's disease."

Liénaux also remarks—

"From the anatomo-pathological point of view, it is interesting to note the abundant ulceration in this horse. Ulceration is rare in Johne's disease in cattle."

From the description given by Liénaux, we should nesitate before accepting this as an undoubted case ot Johne's disease in the horse. Not only had the animal definite tubercular lesions in the mesenteric glands, but the lesions in the intestine appear, from the

description, to be more typical of tuberculosis than of Johne's disease.

Pathological Histology. — Organs for histological examination should be removed from the animal as soon as possible after death, as post-mortem changes rapidly take place. Small pieces of gland and bowel should be placed to harden in methylated spirit, or in formalin-alcohol, or in Müller's fluid containing 10 per cent. of formalin. When one stains for the bacillus, it is preferable to harden in 5 per cent. formalin without the addition of Müller's solution. If Müller's solution is used, the bottles should be placed in a cupboard away from the light, to avoid the precipitation of the bichromate. If alcohol is used, it is advisable to start with a weak solution, and transfer the tissue to solutions of increasing strength, since by this means contraction of the tissue is very largely avoided. After twenty-four hours in the hardening fluid, the pieces of tissue are washed in running water for about the same period of time or longer, and to insure thorough washing, it is best to cut the tissue into thin slices before the commencement of this process. The pieces of tissue are then placed in 90 per cent. alcohol for twelve to twenty-four hours, and, if they are not too thick, the paraffin blocks can be completed on the next day by the following procedure: Place in absolute alcohol for one hour, and then change into fresh alcohol and leave for the same time; then transfer to chloroform, xylol, toluol, or carbon bisulphide, and leave for two hours, the solution being changed at the end of the first hour. Before transferring from the alcohol to the xylol or chloroform, the pieces of tissue may be placed in a mixture of these substances. Now remove from the chloroform, and place into the paraffin bath in a mixture of equal parts of paraffin

and chloroform or xylol, and at the end of an hour transfer to pure paraffin for two hours, the paraffin being changed once. The tissue should then be set in blocks of paraffin, in the ordinary way, by means of two **L** blocks.

The sections should be cut as thin as possible—about 4 μ—a good microtome, such as a Cambridge rocker, being necessary for this purpose. It is best to cut the bowel in a transverse direction at right angles to the surface, in order to obtain a clear view of the villi and their relation to the deeper tissues. Sections, after being treated with xylol and absolute alcohol, are stained by Gram's method, or, better, with carbol-fuchsin and a contrast stain such as methylene blue (Ziehl-Neelsen's method). If stained by the latter method the bacilli are coloured a bright red, in contrast to the tissues, which are coloured blue. Histological changes can be observed better if the sections are stained with hæmatoxylin and eosin.

We have seen that on post-mortem examination the macroscopical appearances of the gut which are especially characteristic are the general thickening of the organ and the great prominence of the corrugations. There is no caseation, and no sign of any ulceration or fibrosis of the tissues, and although in some cases vascular congestion may denote a certain amount of inflammation, this is by no means always present. Thus the post-mortem appearances of the gut lead one to assume that Johne's bacillus does not give rise under natural conditions to an acute inflammation, and microscopical examinations of both early and advanced cases confirm this view.

As we have seen, the alimentary tract may be affected from the duodenum to the margins of the anus, and Johne's bacillus may be found within these

PLATE III.

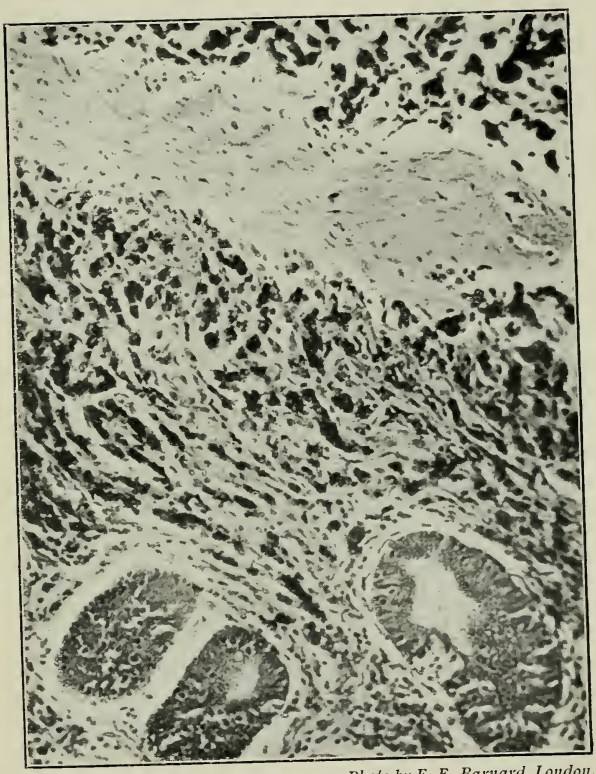

Photo by F. E. Barnard, London.

SECTION OF ILEUM OF COW AFFECTED WITH JOHNE'S DISEASE.

(Magnification about 250 diam.)

The masses of bacilli in the mucous and submucous membranes are black in the plate.

[*To face page* 56.

limits. Sections of badly-affected bowel, stained with carbol-fuchsin, decolorized with 25 per cent. sulphuric acid, and counter-stained with methylene blue, may contain so large a number of bacilli that the section presents a magenta colour to the naked eye. As a rule the number of bacilli present varies directly with the amount of pathological change seen microscopically, and thus they are usually most numerous in and around the ileo-cæcal valve, and it is in this situation that the most marked histological changes are found.

In the small intestines the villi are seen to be swollen and to have a club-shaped appearance, while many of them are flattened on the surface and are stunted. They often contain a large number of clumps of bacilli, mostly at the apex and base, while there is a cellular reaction consisting of epithelioid and round cells and a few giant cells. The bacilli are usually most numerous between the glands and in the lymphoid tissue forming the solitary follicles, but in advanced cases the submucous tissue may be extensively invaded; the bacilli are, for the greater part, in dense clumps, and are both intracellular and extracellular, while they are sometimes found in the characteristic wreath formations. We have not often met with this wreath-like formation of the bacilli, and consider that it is less frequent than is generally supposed. The bacilli seem to spare the gland cells for a long time, as even in advanced cases many of the glands appear to be fairly normal, containing, *perhaps*, an isolated cell here and there full of bacilli; however, the glands may be atrophied to a certain extent, presumably by the mechanical effect of the hyperplasia of the adjacent connective tissue. Between the glands, and deep down in the solitary follicles, there are usually a large number of epithelioid and lymphatic cells and a few giant cells. The last-

mentioned are less frequently found in the intestines than in the lymphatic glands.

Johne and Frothingham, in their original paper, remarked on the scanty number of typical giant cells compared with the quantity usually found in bovine tuberculosis. The increase in thickness of the gut is thus seen to be due to the infiltration of new cells, which are chiefly of an epithelioid type. In advanced cases, in which very many bacilli are present, the normal tissue may be extremely atrophied, while the fibrous tissue stroma remains with an infiltration of epithelioid cells. In cases in which the submucosa is affected, it is found to be thickened generally, while the muscularis mucosæ is always easily distinguishable. The muscular and serous layers of the organ are normal.

Various authors, including B. Bang, have stated that there is some necrosis with desquamation of the epithelium. Bang has since pointed out that this is a post-mortem change, and is not seen in sections of fresh gut that have been hardened in a solution of formalin immediately after the animal is killed. Nodules similar to those found in tuberculosis are rare, and it is probable that true caseation is never found in this situation. In at least 100 carefully examined sections, Johne and Frothingham were able to find only three sites in which there was any similarity to nodules of tubercular disease. These nodules consisted of epithelioid cells and lymphocytes, with one or two giant cells containing bacilli. In this connection the possibility of coincident tuberculosis must be remembered. In the large intestine the condition found is more or less similar to that in the small intestine, but the lesions are usually not so far advanced, and the number of bacilli present is less.

PLATE IV.

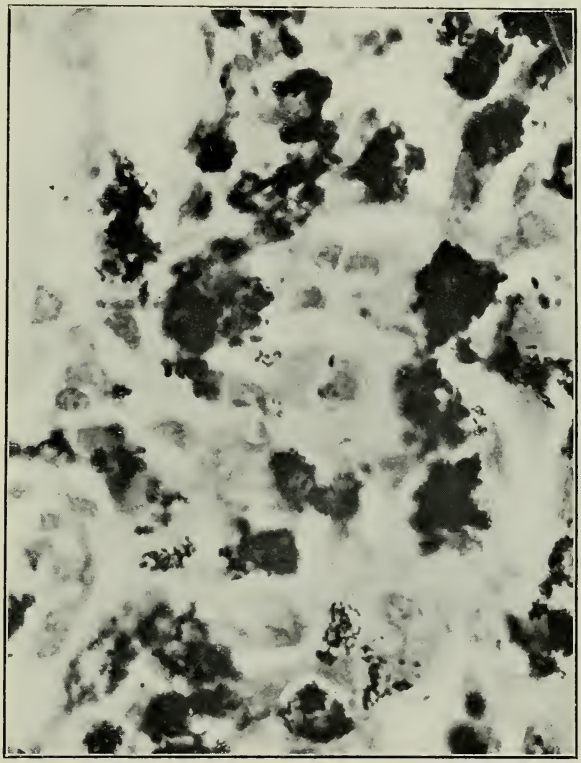

Photo by F. E. Barnard, London.

SECTION OF ILEUM—SAME AS PLATE III.—SHOWING THE LARGE MASSES OF BACILLI IN THE MUCOUS MEMBRANE.

(Magnification about 1000 diam.)

[*To face page* 58.

The extent of the alteration in the bowel seems to bear little, if any, relation to the intensity of the clinical symptoms that are shown by the animal. B. Bang states that in cases in which the clinical symptoms are very marked there is often only a slight thickening of the mucous membrane, and sections show but a trifling infiltration with epithelioid cells. The extent of the pathological changes found in the mesenteric and colic lymphatic glands is usually proportional to the extent of the lesions in the gut; in some cases, however, this is not so, and the glands may be but little affected in a fairly advanced case. The extent of the glandular lesions varies considerably in different cases, and in an advanced stage of the disease the condition of the glands may be œdematous, or may show a considerable destruction of the normal lymphatic tissue.

The usual change found microscopically is an œdematous condition of the organ, the intracellular connective tissue of which is prominent; while the lymphatic cells, instead of being closely packed, as in a normal gland, are somewhat loosely arranged. This is especially marked in the centre of the gland. In many cases no other lesions can be found, and the presence of bacilli, even after a careful search, cannot be demonstrated. In these simple cases, however, it is usual for a small number of bacilli to be present. Sometimes the glands are markedly changed, and numerous epithelioid and giant cells, accompanied by masses of acid-fast bacilli, are found.

It is well to remember that, if an animal dies or is killed shortly after the inoculation of a diagnostic vaccine, microscopical examination of the organs shows an intense congestion of the tissues, especially in the lymphatic glands. This may give rise to an entirely

erroneous conception of the original pathological condition.

The congested vessels of the intestines may take up quite a large proportion of the microscopic field; not only is this so of the submucosa, but also, to a less extent, of the mucous layer. The rest of the organs, with the exception of the liver, appear to be free from bacilli and histo-pathological lesions. In some cases the liver has presented a fatty appearance; but in naturally affected cases we have never been able to find bacilli in this situation, and the condition does not appear to have been recorded by other workers, except by M'Fadyean, Sheather, and Edwards in one cow, and by Stockman, who noted it in an affected sheep. In this connection, the effect of Johne's bacillus on the liver of rabbits, which is described in Chapter IX., is interesting.

The histo-pathological changes found in the intestines of sheep are more or less identical with those found in bovines. In 1908 Vukovic found cases of Johne's disease in sheep in Bosnia, and he was kind enough to send us a paraffin block of the intestine of one of these animals. Very large numbers of acid-fast bacilli were found, the majority being situated in the villi. The general condition of the intestine was similar to that described above in the cases reported by Stockman and by M'Fadyean.

In our experimentally inoculated sheep the liver showed a small round-celled infiltration, and the gland cells of the organ stained badly.

From what has been said it is clear that the pathological condition caused by Johne's bacillus is strikingly different from that found in tubercular disease that has existed for any length of time, although, as M'Fadyean points out, the disease in the early stages is really very

similar to tuberculosis. The absence of caseation (and consequently of ulceration) is one of the most important differences, and, indeed, cellular degeneration can rarely be recognized, except that the nuclei of the cells may lose to a certain extent their power of staining. The general diffusion of the lesions and the absence of any fibrous-tissue capsule are also distinctive features; but perhaps the most prominent difference between this disease and tuberculosis is to be found in the number of causal micro-organisms present. Owing to the comparatively slight effect of a large number of Johne's bacilli on the surrounding cells, many authors have contended that in all probability Johne's bacillus does not secrete toxins in the same way as the tubercle bacillus does. For this and several other reasons, which are discussed in Chapter IX., we agree with this view; but at the same time it may be mentioned that in animals that do not suffer naturally from the disease, and in which the bacilli do not appear to be able to multiply, the caseous peritoneal glands are indistinguishable from tubercular glands macroscopically, and only differ microscopically as regards the number of bacilli and a slighter degree of caseation. This question is considered more fully in Chapter IX.

CHAPTER VI

DESCRIPTION OF JOHNE'S BACILLUS—CULTIVATION OF THE BACILLUS—NATURE OF THE "ESSENTIAL SUBSTANCE"—DESCRIPTION OF CULTURES

Description of Johne's Bacillus.—The specific bacillus of pseudo-tuberculous enteritis, commonly known as "Johne's bacillus," belongs to the acid-fast group of bacteria, and is allied to the various tubercle bacilli. According to the classification of micro-organisms adopted by Lehmann and Neumann, it would be more correct to describe it as a mycobacterium, and the scientific name of the micro-organism would then be *Mycobacterium enteritidis chronicæ pseudotuberculosæ bovis Johne*, the name by which we have suggested it should be known. At the same time, in English literature it is usually referred to as "Johne's bacillus," and in Continental countries as the "paratuberkel-bazillus," or bacillus of pseudo-tuberculous enteritis. In the lesions of the disease the bacilli often appear in extremely large numbers. They are present as slender rods, sometimes slightly bent, and are usually between 1 and 2 μ in length. Sometimes they show a beaded appearance, but this is not so marked as in the tubercle bacilli. Their size and appearance are such that it is not possible to detect any peculiarity by which they can with certainty be differentiated from tubercle bacilli (see Plate V.).

PLATE V.

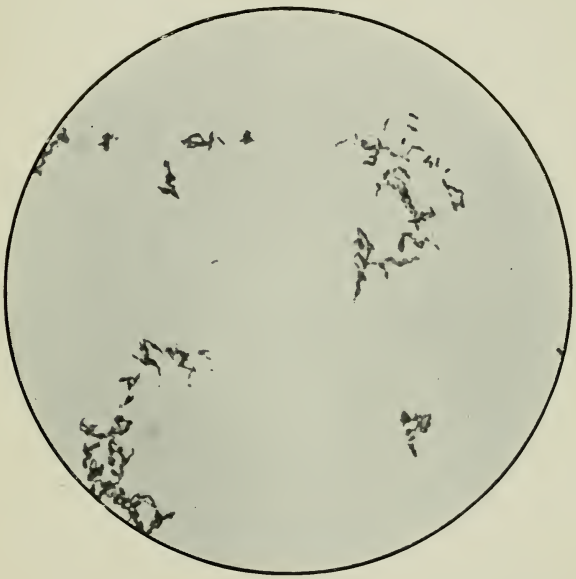

Photo by F. E. Barnard, London.

FILM FROM PURE CULTURE OF JOHNE'S BACILLUS ON GLYCERINE - SALINE - TIMOTHY - GRASS BACILLUS-EGG MEDIUM.

(Magnification about 1000 diam.)

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When first cultivated from the animal body on any of the special media (media containing the dead bodies of other acid-fast bacilli), the bacilli may grow longer and thicker, and lie side by side in a manner very similar to the tubercle bacillus. This is especially so if the medium is a little dry or old, or for some reason is not particularly good. On such media they may attain a length of 4 μ or more, and may show definite dichotomous branching, with club formation, and very distinct beading. When subcultured on to moist and good media, the bacilli soon regain their slender and short form, and in vigorous growing cultures, especially if fluid media is used, they may show little or no beading, and may become very short, appearing almost like cocci.

M'Fadyean, Sheather, and Edwards have noticed "numerous bacilli of quite exceptional length" in smears made from a piece of rectal mucous membrane taken from an animal *intra vitam*. The dimensions of these bacilli are not given, but they are stated to have been much above the average length of Johne's bacillus, and arranged in bundles, in which all the bacilli were approximately parallel. Cultures were made from the tissue on to egg medium containing dead tubercle bacilli, when the bacilli grew; but on ordinary Dorset's egg medium no growth took place. These authors have not observed any definite branching of the bacilli.

Spore formation has not been present in any of our strains, and in none of our cultures have we been able to detect any evidence of motility. In these respects Johne's bacillus agrees with the other members of the acid-fast group.

Holth states that in morphology and staining characters his strain of Johne's bacillus agrees with the description given in our original paper.

As has already been mentioned, Johne's bacillus belongs to the acid-fast group, and is best stained by the method known as Ziehl-Neelsen's. For this process the following solutions are required:

Carbol-Fuchsin.—Fuchsin, 1 gramme; absolute alcohol, 10 c.c. Dissolved and added to 90 c.c. of a 5 per cent. aqueous solution of carbolic acid.

Sulphuric Acid.—A 25 per cent. aqueous solution.

Loeffler's Methylene Blue.—Saturated alcoholic solution of methylene blue, 30 c.c., mixed with caustic potash solution (1 to 10,000 of water), 100 c.c.

The simplest way of staining by this method is to spread a thin film of the suspected material (scraping of the fæces or intestine) on a slide, and dry by gently heating. Then boil a little of the carbol-fuchsin solution in a test-tube, and pour sufficient on the slide to completely cover the film (in place of carbol-fuchsin carbol-gentian violet may be used). To insure that the film remains covered, the slide must be left on a flat bench, and should be stained for three or four minutes. The stain must then be washed off with water, and the film treated with sulphuric acid solution, the washing and acid treatment being repeated until no more of the magenta colour can be extracted. After washing with water, the film should be counter-stained with the methylene blue solution, and this washed off after a few seconds. The film should now be dried, and examined under a $\frac{1}{12}$ oil-immersion lens; it is not necessary to use a cover-glass.

In the case of films made from fæces, it is not easy to extract all the fuchsin from certain portions of the undigested food, but this in no way interferes with the recognition of the bacilli, if present. By this

method of staining, Johne's bacillus is coloured a bright magenta and the rest of the material blue.

When staining films made from pure cultures, counter-staining with methylene blue is not necessary.

Johne's bacillus may also be stained by Gram's method, and is, therefore, Gram-positive. In this method films of bacilli are treated with hot aniline-gentian violet for three or four minutes; the stain is then poured off, and Lugol's iodine solution floated over the surface of the film, and allowed to remain for about a minute; the films are then treated with absolute alcohol until no more colour can be extracted. If examined under the microscope, the bacilli are found to be stained a blackish-violet colour.

From what has been said it is clear that in morphology and staining reactions Johne's bacillus agrees with the various varieties of tubercle bacilli. It shows no character that cannot be met with under certain conditions in the tubercle bacilli.

Cultivation of the Bacillus.—Johne and Frothingham, after making their original discovery of the presence of acid-fast bacilli in the intestinal mucous membrane obtained from cases of pseudo-tuberculous enteritis, attempted to cultivate the bacilli outside the animal body. As a medium they used glycerine-agar, expecting to obtain cultures of some variety of the tubercle bacillus. Their cultures, however, remained sterile, and on microscopic examination they found no evidence of multiplication of the bacilli. In 1904, Stuurman obtained, from a case of Johne's disease at Leyden, a pure culture of an acid-fast bacillus, and again, in 1908, from the inguinal gland of a guinea-pig inoculated with a small piece of the intestine of an animal affected with Johne's disease he obtained another culture, which showed characters similar to the first. These

cultures, which were isolated on glycerine-serum, glycerine-agar, and other media, some of which contained decoctions of the grass *Phleum pratense*, were submitted to Koch and Rabinowitsch, who declared them to be avian tubercle bacilli. Inoculation experiments with these cultures failed to produce Johne's disease, but caused lesions in the small laboratory animals which proved them to be the avian type of tubercle bacillus. Mettam, in a private communication, states that he has also isolated a culture of the avian tubercle bacillus from a case of Johne's disease. We consider these results to be secondary infections with the avian tubercle bacillus. This view is supported by a large number of authorities, including Bang, Miessner, M'Fadyean, Markus, and Meyer, who, previous to the year 1911, experimented with infected material from cows, and failed to obtain cultures of the specific bacillus. It will be shown, also, that when the bacillus has been isolated on the special medium, it can be subcultured on ordinary laboratory media only after prolonged cultivation and acclimatization outside the animal body, which proves definitely that the bacillus is not a variety of the avian tubercle bacillus.

In 1908, Bugge and Albien published a short note to the effect that they had succeeded in obtaining a pure culture of Johne's bacillus (*Paratuberkelbazillen*) from the mesenteric gland of an affected animal. No particulars as to the medium were given in this paper; but in 1910, Albien published a further note giving the constitution of the medium used. This was as follows:

Nährstoff Heyden	5–10
Agar	10–20
Glycerin	20–30
Kochsalz	5
Normallösung von Kristallsoda (28 : 100) ...	5
Aqua dest	1,000

Hesse, in 1899, first tested *Nährstoff Heyden*, which is a preparation of egg albumen, for the cultivation of the tubercle bacillus. On the medium given, Albien states that he obtained a growth on eight tubes out of several hundred inoculated; but the cultures were not tested on calves, and no confirmation of the results has been published. Moreover, our own attempts to isolate and grow Johne's bacillus on such a medium have given uniformly negative results.

In 1909, Melvin of America stated that he had obtained a luxuriant growth of an acid-fast micro-organism from a case of chronic bacterial dysentery (Johne's disease) occurring in Oregon. The culture was made on an egg medium, but the bacillus was overgrown by a saprophytic micro-organism, and no subculture could be made. As it is stated in the Report of the Bureau of Animal Industry for 1910 that attempts to grow Johne's bacillus had failed, it seems clear that the acid-fast bacillus grown was not Johne's bacillus, and we know from more recent experiments that the bacillus gives no growth on a simple egg medium.

Early in 1910 we started some experiments with the object of cultivating Johne's bacillus, and of preparing a diagnostic vaccine from the culture obtained. A preliminary note on the results of this work was included in a paper by one of us on the cultivation of the lepra bacillus of man, published in 1910. In June of that year we obtained from Mr. De Vine, of Birmingham, a specimen of the bowel and mesenteric glands of a cow suffering from Johne's disease. The ileum showed well-marked lesions of pseudo-tuberculous enteritis, and films made from the gut revealed the presence of a large number of acid-fast bacilli. The specimen was fresh, and cultures were made

immediately on to all the ordinary laboratory media. In our experiments the diseased tissue was placed either directly on to the medium to be tested or after previous treatment with a 1 per cent. watery solution of ericolin to kill contaminating micro-organisms. The method of making direct cultures was as follows: The gut and glands were thoroughly washed with water, and the surface of an infected area seared with a hot spatula; microscopic films were made from the tissues beneath the part seared to prove the presence of the specific bacillus. Small pieces of tissue were then removed with sterile scissors and rubbed over the surface of the culture medium to be tested. If the tissues were contaminated, the indirect method of cultivation was adopted—*i.e.*, the tissue was previously placed in a sterile 1 per cent. watery solution of ericolin and heated for an hour at 37° C., after which the particles of tissue were removed and rubbed over the media to be tested. The ericolin was used to kill off most of the contaminating micro-organisms, for, as has already been pointed out by one of us (F. W. T.), the acid-fast group of bacilli are but little affected by the action of this substance.

In the first instance we used as media unheated extracts and tissues of normal cattle. The fresh extracts of glands and organs, including the intestines, were made and sterilized by passing through a porcelain filter. These extracts were placed in sterile tubes, and each was tested as a medium *per se*, and also added in various proportions to the ordinary laboratory media. Small sterile portions of bovine organs, especially lymphatic glands, were also obtained and placed in sterile tubes. All these media were tested in various combinations, with and without glycerine, cholesterine, various sugars, fresh blood,

and other substances. The cultures were placed in an incubator at 39° to 40° C. On none of these media could any definite growth of the bacillus be detected.

With the object of testing for the possible presence of an ultramicroscopic virus working in symbiosis with Johne's bacillus, we prepared an extract of the infected intestine of a cow suffering from Johne's disease and passed it through a Doulton white filter. The sterile filtrate so obtained was added to various media, and the whole series inoculated with small portions of intestine affected with Johne's disease. These media all gave negative results.

From the experiments conducted on this case we came to the conclusion arrived at by most other workers, namely, that the specific bacillus would not grow on any artificial medium known to bacteriologists, and that if successful cultivation were to be achieved, some new medium would have to be prepared. We considered also that the failure of growth of the specific bacillus must be due, either to the poisonous action of some substance in the medium, or to the absence of some foodstuff or stimulant necessary for its vitality and growth.

At the same time we were struck by the apparent close relationship existing between this micro-organism and the tubercle bacillus; and as the bacillus of pseudo-tuberculous enteritis and the tubercle bacillus both grow in the same species of animal, we thought it highly improbable that there could be any substance in the ordinary laboratory media which would act as a poison to the one bacillus and not to the other. This possibility was accordingly excluded, and we were forced to conclude that the failure to grow the bacillus must be due to the absence of some necessary food-stuff.

Considering again the apparent close relationship between the tubercle bacillus and the bacillus of pseudo-tuberculous enteritis, and the fact that both these bacilli live in the bodies of bovines, we judged it probable that they would require the same chemical substances for building up their protoplasm, certain of which substances could be elaborated from artificial media by the tubercle bacillus but not by the bacillus of pseudo-tuberculous enteritis—in other words, that the latter bacillus has lived a pathogenic existence from such remote ages that it has lost the original power of its wild ancestor—whatever bacillus that may have been—and can no longer build up all its necessary foodstuffs outside the animal body.

It was thought probable that if these substances could be obtained ready formed, and were added to some good artificial medium (Dorset's egg medium) the bacillus would grow, and, further, that these substances might be elaborated by allied micro-organisms such as the tubercle bacillus, and even stored up as reserves in their envelopes. On this reasoning, which led to a partially successful cultivation of the lepra bacillus of man, we decided to prepare media containing these allied bacilli, which had been killed by heat.

We had, at the time, in our possession about three hundred strains of tubercle bacilli, mostly isolated from human tuberculous material on Dorset's egg medium. Some of these cultures were taken, and after the necessary subcultures had been made, they were killed by steam. The growth was then scraped off, care being taken to avoid any admixture of the medium which might contain the waste products of bacillary growth and be toxic to the bacillus of pseudo-tuberculous enteritis; more recently we have found this precaution to be unnecessary. The growth of tubercle bacilli thus

obtained was ground in a mortar with glycerine and saline, steamed for half an hour, and added to the white and yolk of new-laid eggs in the following proportions: Egg, 75 parts; 0·8 per cent. sodium chloride in re-distilled water, 25 parts. These were thoroughly mixed, and to the mixture were added tubercle bacilli 1 per cent. and glycerine, 5 per cent. The medium was placed in sterile test-tubes; these were plugged with cotton-wool, and heated in a hot-water bath at 60° C. for one hour on three successive days, the tubes being incubated at 37° C. in the intervals between steaming. Finally the tubes were inspissated in slopes at 85° to 90° C.

A second case of pseudo-tuberculous enteritis was now obtained from Mr. De Vine. Specimens of intestine and glands were received on July 28, 1910. Both the intestine and glands showed the typical characters of the condition, and large numbers of Johne's bacilli were present in various parts of the tissues. Unfortunately, owing to the hot weather prevailing at the time, the specimens on delivery had commenced to decompose; but, in spite of this, we prepared some cultures in the manner previously described, both directly, and indirectly after treating with ericolin solution. The cultures were made on several of the media tested with the first cases, as well as on a number of tubes of the special tubercle bacillus medium. The tubes were capped with gutta-percha tissue, and incubated at 39° to 40° C. After two days' incubation all the direct tubes were badly contaminated, yet those inoculated with ericolinised material showed only a few contaminating colonies. Sub-cultures were made from uncontaminated areas of most of the latter tubes on to fresh tubes of the same medium; but, owing to the small amount of the

tubercle bacillus medium then prepared, only one of these tubes was subcultured. This one was made from a gland. Films from these subcultures were examined microscopically at intervals of about four or five days. After nineteen days the subculture on the special medium showed quite definite evidence of multiplication; the bacilli had grown larger and thicker; they were well stained, and were present in large, close masses. Subcultures were made from this tube on to fresh tubes of various media, including one tube of the special tubercle bacillus medium. These were examined at intervals as before, and the subculture on the special medium showed microscopic evidence of growth in ten days. Both the first and second subcultures showed growth visible to the naked eye after four weeks, and this gradually increased, reaching a maximum in about eight weeks.

These tubes were easily subcultured on to fresh tubes of the same medium, but on none of the ordinary laboratory media were we able to get any evidence of growth.

The third case of pseudo-tuberculous enteritis was obtained from Mr. Hamilton. Specimens of intestine, but no glands, were received on September 23, 1910. They showed the typical lesions of the disease, and a very large number of Johne's bacilli were present in the tissues. When delivered, the specimens had already commenced to decompose; but from them cultures were made as previously described, both directly, and indirectly after treatment with ericolin solution on various media, including tubes of the special tubercle bacillus medium. The tubes were capped with gutta-percha tissue, and placed in an incubator at 39° to 40° C. The results were the same as in Case 2; all the direct tubes were badly contami-

nated, and those tubes which had been inoculated with material previously treated with ericolin solution grew only a few contaminating colonies. Of the latter the cultures on the tubes of special medium were subcultured from uncontaminated areas on to a number of fresh tubes of various media, including the special medium. The subcultures on the ordinary media remained sterile, but those on the tubercle bacillus medium grew Johne's bacillus in pure growth, and were without difficulty subcultured on to fresh tubes of the special medium. Naked-eye evidence of growth was present in the first subcultures after about six weeks.

The fourth case was obtained from Mr. De Vine, a specimen of the intestine being received at the Institution on January 26, 1911. It showed the typical lesions of pseudo-tuberculous enteritis, and a large number of the specific bacilli were present in the lesions. Since the specimen was quite fresh, cultures were made, as previously described, from the ileum, cæcum, and ileo-cæcal valve, directly on to nine tubes of the special tubercle bacillus medium; these were capped, and placed at 39° to 40° C. After three weeks' incubation two tubes were found to be contaminated, whilst the remainder were covered with extremely minute colonies of Johne's bacillus, and showed no contaminations. The cultures grew well, and were subcultured without any difficulty on to the special medium. Subcultures made on to Dorset's egg medium, glycerine-agar, and various other media gave no growth.

Case 5 was obtained from Mr. Hamilton, and was received at the Institution on February 8, 1911. The specimen, consisting of ileum and ileo-cæcal valve, showed the typical lesions of pseudo-tuberculous

enteritis, and a considerable number of acid-fast bacilli were present in the lesions. Cultures were made from several parts of the specimen directly on to twelve tubes of Dorset's egg medium. They were taken in the manner already described, but as the specimen was fresh on arrival, previous treatment with ericolin solution was unnecessary. The tubes were capped with gutta-percha tissue, and placed in the incubator at 39° to 40° C. On the following day they were examined, and found to be free from contaminating colonies, so the tiny pieces of tissue were removed from three of these tubes and placed on to three tubes of the special tubercle bacillus medium. These were capped, and placed in the incubator with the other tubes at 39° to 40° C. Six weeks later the three tubes of special medium showed a few tiny colonies of Johne's bacillus. Compared with the previous cases the rapidity of growth was very slow, and was slight in amount, due, as was proved later, to the unsuitability of the particular strain of tubercle bacillus incorporated in the medium. Subcultures from these tubes on to tubes of a fresh batch of tubercle medium grew well. All the original cultures on Dorset's egg medium remained sterile, as also did subcultures from the special medium on to Dorset's egg medium.

The experiments with these cases (2 to 5) prove definitely that it is possible to isolate from the intestines of cattle suffering from Johne's disease an acid-fast bacillus which will grow on a medium containing the dead bodies of human tubercle bacilli, but will not grow on any of the ordinary laboratory media.

Slight modifications in the constitution of the medium were now made, and it was found that media containing about 4 per cent. of glycerine yielded the best results.

Growth, however, took place with 10 per cent. of glycerine, and in the presence of a much higher percentage of salt; but in the complete absence of glycerine growth was not nearly so good. It was found better to dry the tubercle bacillus after killing it, and before making it up into medium. This was done *in vacuo* over caustic potash. The improvement in this case may have been due to the formation of breaks in the continuity of the covering of the bacillus, thus allowing the "essential substance" to diffuse more easily into the medium. Of the dried bacilli, ½ to 1 per cent. was found to be sufficient to add to the egg medium. Alterations in the normal alkalinity of the egg medium produced no good results. The bacillus seems to prefer a slightly alkaline medium.

In a further series of experiments the dead tubercle bacilli were added to ordinary glycerine-agar and glycerine-broth, but these media did not, as a rule, give such good results, though glycerine-peptone-beef broth, containing ½ to 1 per cent. of dried tubercle bacilli, gave a fairly satisfactory growth. In the case of the subcultures on the agar medium, growth occurred along the needle-track as small, white, elevated colonies, which at first were smooth and discrete, but after six or eight weeks they coalesced and presented an irregular surface. When older, the growth turned a dull yellowish-white, and in tubes with a single colony this spread and became more heaped up in the centre, while the margins were thinner and irregular. The growth, although slow, was very similar to that of the tubercle bacillus. The growth in the broth subcultures occurred as tiny granules at the bottom of the flask, and these little masses of bacilli increased in size and number, though very slowly. At the end of six months one flask showed a very thin film on the surface of the

medium. This slowly increased, spreading over the surface of the medium and becoming more heaped up. The colour was a dull white, slightly tinged with yellow. These broth cultures were used for preparing our first series of diagnostic vaccines.

We now proceeded to test our strains of Johne's bacillus on media in which the dead tubercle bacillus was replaced by various other micro-organisms. We soon found that some strains of human tubercle bacilli were more suitable than others; and, further, that if the human tubercle bacillus was replaced by the bovine type, no growth of Johne's bacillus took place, and that this was so even when subcultured from strains that had been growing outside the animal body for a year. Several strains of tubercle bacilli isolated from cats were also tested, but gave negative results. The cat strains were distinctly bovine in character, and, as was the case with the other tubercle bacilli, they were grown on Dorset's egg medium. (A discussion of these results and some details of further experiments with the bovine strains are given later.)

In view of Johne and Frothingham's original suggestion that the bacillus of pseudo-tuberculous enteritis was either an avian tubercle bacillus or some variety of it, and the support this view received from the reactions to avian tuberculin obtained by Bang in animals suffering from Johne's disease (*vide* Chapter VII.), the avian tubercle bacillus naturally suggested itself for incorporation in media. Two strains, therefore, were tested; one was obtained from Kral, and the other isolated by ourselves from a natural case of tuberculosis in a hen. The bacilli were grown on large tubes of Dorset's egg medium, and when the growth had reached a maximum it was killed, scraped off, dried, and made up into medium as already described for the

human type. On this medium our strains of Johne's bacilli usually grew, but only to a slight extent, and the medium proved to be quite unsuitable for practical purposes.

From the fact that since the remotest times the timothy-grass bacillus (*Bacillus phlei* Moeller) found on *Phleum pratense* must have been continually ingested by ruminants in their food, the possibility of this bacillus being the wild ancestor from which Johne's bacillus has arisen is at once obvious, and if such be the case one might expect, from what has been already said, that *B. phlei* would be a very suitable variety to use in preparing the medium. To the egg medium, therefore, this bacillus, grown on ordinary broth, was added to replace the human tubercle bacillus, ½ to 1 per cent. of the dried bacillus being used. Johne's bacillus on this medium grew quickly and well, the growth being better than on any of the media so far tried. A slight growth was visible along the needle-track in one week, and in six weeks the growth closely resembled that of a bovine tubercle bacillus freshly isolated from the animal body.

In exactly the same way other acid-fast bacilli were tested—*i.e.*, incorporated, when dead and dried, in Dorset's egg medium. The following were experimented with:

1. The smegma bacillus of Moeller.
2. The nasenschleim bacillus of Karlinski.
3. The fish tubercle bacillus of Dubard.
4. The butter bacillus of Rabinowitsch.
5. Bacillus from urine, Marpmann.
6. Pseudoperlsucht bacillus of Moeller.
7. Bacillus from butter, Grassberger.
8. Three of the Tobler group of acid-fast bacilli; Tobler, I., IV., and VI., Kral.

Johne's bacillus, when inoculated on to media containing Nos. 1, 2, 5, 6, and 7, gave a good growth; but on the rest growth was usually absent, though a very slight film was occasionally present on the media containing some of the Tobler varieties.

A number of blastomyces, streptothrices, cocci, and bacilli that are not acid-fast, were also tested, but no growth was obtained on media containing any of these micro-organisms. In all such experiments we used strains of Johne's bacillus that had been isolated on the tubercle bacillus-egg medium, and that had been growing outside the animal body for a considerable time, and it was thought that the good growth obtained with *B. phlei* medium might be due to the fact that our strains were becoming acclimatized to artificial culture media. To test this point we made primary cultures on to a *B. phlei* medium. A further specimen of diseased gut was obtained through the kindness of Mr. De Vine. In this case the disease was in a very early stage, and the thickening of the intestines was only quite moderate. Films were made from the ileum and ileo-cæcal valve, but only a very few acid-fast bacilli could be found after a prolonged search. Cultures, however, were made from the regions showing most bacilli, on to tubes of Dorset's egg medium, and also on to tubes of *B. phlei* medium. After five weeks' incubation there was no evidence of growth on Dorset's egg medium, but on the *B. phlei* medium definite evidence of multiplication was observed. Subcultures from the *B. phlei* medium on to fresh tubes of the same medium grew well, as also did those on a medium containing the human tubercle bacillus, but on ordinary laboratory media there was no growth.

The bacillus isolated resembled in every way the bacilli isolated from the four previous cases, and the

cultural characters were also the same. More recently we have isolated Johne's bacillus on media containing some of the other acid-fast bacilli mentioned above —*i.e.*, Nos. 1, 2, 5, 6, and 7.

From these experiments it is clear that Johne's bacillus will grow on media containing the dead timothy-grass bacillus, or bacilli Nos. 1, 2, 5, 6, or 7, not only after it has been cultivated in the laboratory for a considerable period, but also when taken direct from the diseased gut of cattle.

Having determined the various acid-fast bacilli most suitable for the growth of Johne's bacillus, we attempted to extract the "essential substance" (the substance necessary for the growth of Johne's bacillus) from certain of these bacilli. The timothy-grass bacillus was chosen, chiefly because it gave the best results in the experiments with the whole bacilli, also because it is harmless to man, and grows quickly on simple media, thus enabling a large quantity to be obtained in a short time.

For this purpose Dr. W. Bulloch kindly gave us a quantity of this bacillus, besides various dead and dried tubercle bacilli, which had been given to him by Professor Bang about eight years previously. Many of these had already been extracted by Bulloch and Macleod when investigating the acid-fast properties of the tubercle bacillus. In a preliminary series of experiments the different bacillary powders were made up into media, the tubercle bacillus of our original medium being replaced by one or other in quantities of $\frac{1}{2}$ per cent. Tubes of each were inoculated with a fresh culture of Johne's bacillus, and the results may be summarized as follows:

Dried timothy-grass bacillus (*B. phlei*) gave very good results.

Dried human tubercle bacillus gave good results, but inferior to *B. phlei.*

Dried bovine tubercle bacillus gave negative results.

Dried swine tubercle bacillus gave negative results.

Dried tubercle of uncertain source, freed from wax and fat, gave negative results.

Dried tubercle of uncertain source, freed from wax, fat, and proteid, gave negative results.

The timothy-grass bacillus and the human tubercle bacillus were found to be equally good when previously autoclaved in normal saline for thirty minutes at 120° C. The results proved conclusively that the "essential substance" contained in these bacilli is comparatively stable, remaining undiminished in the timothy-grass bacillus and the human tubercle bacillus that had been killed and dried eight years previously, and also after they had been autoclaved. It is interesting to note that the bovine type, when added to media, again gave negative results, and that the bacillus of uncertain source, which had been extracted with acid-alcohol, etc., also failed to give any growth with Johne's bacillus.

We now took 1 gramme of the dried *B. phlei* powder, and extracted with 20 c.c. of 0·8 per cent. sodium chloride in redistilled water containing 4 c.c. of glycerine. The mixture was autoclaved for half an hour at 120° C., and then filtered. The filtrate was added to the yolk and white of hens' eggs in the proportion of 1 part of filtrate to 3 parts of egg. The residue was washed several times with normal saline, dried, and made up into medium with egg, so that ½ per cent. of the residue was present. The same process was followed with another gramme of the

B. phlei, except that redistilled water was used in place of the glycerine and saline for extraction. We found that Johne's bacillus grew on the glycerine-saline extract medium, and on that containing the residue. It also grew on that containing the residue after extraction with redistilled water, but it failed to grow on the medium containing the distilled-water extract. From this we concluded that the "essential substance" is only very slightly, if at all, extracted by redistilled water, but that it is soluble in a glycerine-saline solution, although in the above case it is clear that some of it remained in the residue.

A further series of experiments was made, using ethyl alcohol as the solvent. Two grammes of dry, powdered *B. phlei* were placed in a Soxhlet apparatus with 100 c.c. of absolute alcohol and extracted for three hours. The residue was dried in an incubator, and the alcoholic extract evaporated to dryness, leaving a dark yellowish, sticky mass. The extract and residue were then weighed separately, and it was found that the bacilli were reduced in weight from 2 grammes to 1·25 grammes, the difference being represented by the extract. (In later experiments we have extracted very much larger quantities of various acid-fast bacilli, sometimes using as much as 100 grammes of dry bacilli, and we have found that the proportion of extract to residue varies considerably, the proportion of extract usually being lower than that given above.) Media were prepared with the residue and with the extract thus obtained: some contained only $\frac{1}{4}$ or $\frac{1}{2}$ per cent., and some 1 per cent., of the extract or residue. Tubes of each were inoculated with a young growth of Johne's bacillus and incubated at 39° C. Good growths were obtained on

all the media containing the extracts, but as a rule there were none on the residues.

These experiments proved that the substance in the timothy-grass bacillus and in allied bacilli that is essential for the growth of primary cultures of Johne's bacillus is extracted by hot ethyl alcohol. As is well known, if this hot extract is allowed to cool, a yellowish sticky sediment and a white flocculent precipitate of wax, etc., form, and can be removed by filtration. The clear-coloured filtrate, when evaporated to dryness, leaves a thick oily residue, which becomes firmer on cooling. Part of this residue is soluble in hot and in cold chloroform, leaving an insoluble portion, part of which floats on the surface of the chloroform. This can be removed by filtration, and, unlike the chloroform soluble portion, it is found to be readily soluble in water.

Each of these portions was now tested in media, and all gave positive results with Johne's bacillus, the best being that which is insoluble in chloroform (see Plate VI.).

Our researches with regard to media for the cultivation of Johne's bacillus had reached this stage when the results were communicated to the Royal Society (November 5, 1911), and, with some animal inoculation experiments and the results of certain vaccine tests to be discussed later, formed the subject of the Brown Lectures given at the Royal College of Surgeons early in January, 1912.

In May, 1912, Halfdan Holth, working in the laboratories of Professor Jensen in Copenhagen—to whom, early in 1911, we had sent cultures of Johne's bacillus —published an account of experiments with this bacillus. (Results communicated March 21, 1912.)

In March, 1911, from a natural case of the disease in a Jersey cow which had reacted to avian tuberculin,

PLATE VI.

FIG. 1. FIG. 2. FIG. 3.

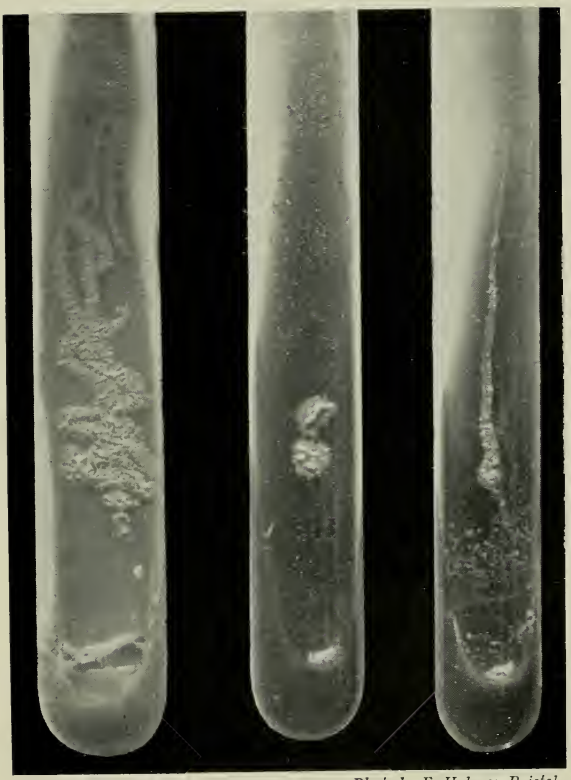

Photo by F. Holmes, Bristol.

CULTURES OF JOHNE'S BACILLUS.

FIG. 1.—Streak culture on an egg medium made up with saline and containing 4 per cent. glycerine and ¼ per cent. of the chloroform insoluble portion of the cooled alcoholic extract of *B. phlei*.

FIG. 2.—Isolated colonies on a glycerine-agar medium containing dog's filtered ascitic fluid and the same extract of *B. phlei* as Fig. 1.

FIG. 3.—Streak culture on same medium as Fig. 2.

[*To face page* 82.

Holth inoculated the following media, on which he obtained good growths:

(*a*) Blood-serum (horse) with 4 per cent. glycerine and 2 per cent. dead tubercle bacilli added. The bacilli were obtained from a two months' old broth culture, and, before being added to the medium, were mixed with a small quantity of salt solution and heated to 100° C. for one and a half hours. The bacilli were of the human type.

(*b*) Blood-serum with a quarter volume of liver broth, 2 per cent. dead tubercle bacilli, and 4 per cent. glycerine.

(*c*) Serum agar with 2 per cent. dead tubercle bacilli and 1 per cent. glycerine.

(*d*) Egg medium as used by ourselves.

The tubes were sealed with paraffin and placed at 37° C., and in six weeks multiplication of the bacilli was found to have taken place.

Holth found that the best growth occurred on (*b*). At the end of six weeks the whole surface of the medium was covered with colonies visible to the naked eye and varying in size from $\frac{1}{2}$ to 1 millimetre. Sub-cultures on to other similar media showed good growth after four weeks. To avoid the presence of tubercle bacilli in the medium, a glycerine extract was prepared. The bacilli from the growth on about 200 c.c. of a two months' broth culture were mixed with about 40 c.c. of glycerine, and, after shaking, the mixture was heated for an hour at 100° C. After standing at room temperature for several days, the pale yellowish-brown liquor was decanted. The addition of 5 per cent. of this liquid to the media in place of the tubercle bacilli was found to give good results.

Holth states in his paper that the bacilli cultivated agreed in their morphology and staining reactions with

the cultures obtained from us. In liver broth to which blood-serum and the glycerine extract mentioned above had been added, Holth observed, after an interval of about four weeks, a slight growth which appeared as a sediment at the bottom of the tubes. This growth gradually increased in amount, and later formed a film on the surface of the medium. The surface growth was often observed to be thick and knobby. Attempts to subculture the bacillus on to media not containing extracts of tubercle bacilli or their dead bodies were unsuccessful, with the possible exception of serum-agar containing 1 per cent. of somatose and 1 per cent. of Heyden's "Nährstoff," on both of which a slight growth might have been detected.

Regarding the addition of serum to the medium as advocated by Holth, we found that it did not improve our original egg medium, so performed no further experiments on these lines until after the publication of Holth's paper, when we tested dog's ascitic fluid with liver broth-agar containing an extract of *B. phlei*. The unheated ascitic fluid was added just before setting the agar, and for one batch the fluid was first passed through a Doulton porcelain filter. This series of experiments showed the agar to be improved by the addition of the fluid, especially when it was filtered before being added to the agar. With the addition of blood-serum, however, we did not obtain such good results, but this may have been due to the fact that we used rabbit serum, while Holth used horse serum.

Since Holth's paper, M'Fadyean, Sheather, and Edwards have published similar results. These workers isolated the bacillus from twenty-three naturally infected cases, and from four experimentally inoculated

animals, and this they did on media containing the dead bodies of acid-fast bacilli, glycerine extracts of such bacilli, or the products of their growth. These authors prefer an agar or a serum-agar foundation for their media as being less troublesome to prepare than egg media. They were unable to obtain growth on any of the ordinary media, but they obtained good growths on liquid media made up of two parts of ordinary broth containing 5 per cent. glycerine, and one part of a 5 per cent. glycerine-broth culture of the *B. phlei* that had been steamed for two hours, and then centrifuged to remove the bacilli. Their cultures on various media are illustrated by excellent photographs in the *Journal of Comparative Pathology*, September, 1912.

Regarding the use of a glycerine extract of the tubercle bacillus for making media, the authors state :

> "As previously stated, at the outset we used the egg medium first recommended by Twort and Ingram, and nearly always with success. It is, however, a medium with some notable defects. In the first place it is a little troublesome to prepare, especially when perfectly fresh eggs are not available. Secondly, it is an opaque medium, and therefore does not allow one to observe the appearance of any growth on it by transmitted light. On this account peculiarities of growth are apt to be overlooked, and accidental impurities are not so easily detected as on a transparent or translucent medium. Finally, the abundant presence of tubercle bacilli in the substance of the medium is very objectionable when in doubtful cases one wishes to determine by microscopic examination whether any multiplication of the implanted Johne's bacilli has taken place.

"For these reasons, and quite independently of Holth's attempts in the same direction, we endeavoured to obtain some other medium for the cultivation of the bacilli.

"As it appeared to be probable that the essential substance present in the mixture of egg, tubercle bacilli, and glycerine was something extracted from these bacilli by the glycerine, it was resolved to endeavour to obtain a strong glycerine extract of tubercle bacilli, which when added to agar or other transparent medium might render that suitable for the growth of Johne's bacilli.

"The dead bodies of tubercle bacilli cultivated on the surface of 5 per cent. glycerine broth were therefore extracted with hot glycerine, and after removal of the bacilli the glycerine extract was added to various media, which were then inoculated with Johne's bacilli. This immediately yielded positive results, and enabled us to obtain good growths on media that were at once transparent and free from tubercle bacilli.

"For purposes of extraction various strengths of glycerine were employed, but a better extract appeared to be obtained with diluted glycerine than with the pure substance."

In our paper (*Royal Society*, B. 84, 1912, communicated November 7, 1911), to which the authors in their paper have frequently referred, we do not suggest that media *must* be made of egg and tubercle bacilli; various modifications are discussed, including those mentioned by M'Fadyean, Sheather, and Edwards. On p. 525 we stated:

"Experiments showed that ½ to 1 per cent. of the dried tubercle bacillus was the most suitable

quantity to add. To obtain the best results, the dried bacilli should be ground up with the glycerine which has been mixed with an equal quantity of 0·8 per cent. saline, and the remainder of the saline added later. The emulsion so obtained should then be steamed for fifteen minutes, and, when cool, added to the egg. The probable explanation for this is that the glycerine acts as a solvent for the essential substance, and some experiments to be described later tend to confirm this suggestion."

On p. 529 we discussed these later experiments thus:

"Some further experiments were now made: 1 gramme of dried timothy-grass bacilli was taken and extracted with 20 c.c. of 0·8 per cent. sodium chloride and 4 c.c. of glycerine. The mixture was autoclaved for half an hour at 120° C. and passed through filter-paper. The filtrate was then added to the white and yolk of hens' eggs in the proportion of one part of the filtrate to three parts of egg. Another batch of medium was prepared by taking the residue of the timothy-grass bacillus, washing it repeatedly with normal saline, filtering it, and drying the residue. This residue was made up into medium, the tubercle bacillus of the original tubercle egg medium being replaced by ½ per cent. of the residue of the timothy-grass bacillus. . . . We found that Johne's bacillus grew well on the medium containing the glycerine saline extract and on that containing the residue."

On p. 525 we also state:

"In another series of experiments the egg was replaced by various other substances, such as

broth or agar. These, as a rule, did not give such good results, although ordinary glycerine-peptone-bouillon, made distinctly alkaline and containing ½ to 1 per cent. of dried tubercle bacilli, gave a fairly satisfactory growth. This, with other experiments to be described later, proved that Johne's bacillus can grow quite well in the absence of albumen."

In the same paper, p. 537, we wrote:

"As has been stated, no growth occurs on any of the artificial media in general bacteriological use, such as peptone-bouillon, agar, gelatine, serum, potato, or egg, even when such substances as glycerine, sugars, amino-acids, fresh blood, etc., are added. It is absolutely essential that certain previously detailed bacteria or extracts from them be added to one or other of the media, before any growth of Johne's bacillus takes place, and this is equally true for strains of Johne's bacillus which have been freshly isolated from the animal body, and for stains which have been cultivated on artificial media for fifteen months or more."

From the quotations given it will be seen that the question of obtaining a clear medium and one free from the bodies of tubercle bacilli, or allied bacilli, was fully considered in the paper to which we have referred, and, as we also pointed out, the "essential substance" in these bacilli can be extracted more efficiently by hot alcohol than by glycerine if a Soxhlet's apparatus is used when extracting with the former. In another part of their paper M'Fadyean, Sheather, and Edwards, mention some of these experiments.

In their paper (*Journal of Comparative Pathology*, vol. xxv., part iii.) M'Fadyean, Sheather, and Edwards

also give an account of experiments carried out to test the relative value of the human, bovine, and avian types of tubercle bacilli. On pp. 267-268 they state:

"Twort and Ingram found that the best growth was obtained on Dorset's egg medium to which timothy bacilli (bacillus phlei) had been added, but apparently tubercle bacilli of the human type were in this respect only slightly inferior. On the other hand, they obtained little or no growth on media with which the avian type of tubercle bacillus had been incorporated, and the results were entirely negative when bovine tubercle bacilli were employed.

"Our results are not at all concordant with these. In comparative experiments we have generally found that tubercle bacilli of the avian type gave the best results, and that human bacilli were slightly superior to the bacillus phlei. The word 'generally' has been used in the preceding sentence because absolutely uniform results are not obtainable with different lots of media prepared in the same way, and it has therefore occasionally happened that no difference could be observed between parallel cultures according as the medium contained the one or the other kind of acid-fast bacillus. The most striking difference of opinion, however, has arisen in connection with the use of tubercle bacilli of the bovine type. As stated above, Twort and Ingram found this type of organism useless for the purpose in view, and they appear to have thought that in this fact they had discovered evidence contrary to the view that the human and bovine types are only slightly different varieties of one and the same organism. We have found that

the bacillus of Johne's disease can be cultivated, either as primary or as subcultures, on media to which bovine tubercle bacilli or extracts prepared from them have been added, and our results would not justify us in stating that in this respect the bovine type is inferior to the human type or to the bacillus phlei. And it ought to be stated particularly that our successes have been obtained with quite typical bovine bacilli, isolated directly from bovine lesions by ourselves, markedly dysgonic, and proved by experiment to be virulent for bovine animals and rabbits."

The results obtained with the bovine type by these authors are certainly different from those obtained by ourselves, and given in our paper in the Proceedings of the Royal Society and later in the *Centralblatt für Bakteriologie.* The different results are probably accounted for in part, at least, by the difference in the media on which the bovine tubercle bacilli were grown. Experiments bearing on this point are described later in this chapter.

The authors (M'Fadyean, Sheather, and Edwards), however, appear to have misunderstood our remarks on the relationship of the human and bovine types of tubercle bacilli. We made an observation incidentally on this point, and suggested that the differences we had found were worthy of further investigation. At the same time we pointed out that we did *not* consider that the different results we had obtained with the two types represented an important *biological difference*, but that the difference in the bacilli was probably *physiological* in nature. Our remarks were:

"Whatever this difference between the two types of bacilli may be due to, it does not in our

opinion necessarily represent an important biological difference: it is probably physiological in nature, and may be due to the presence or absence of some reserve food material existing, or otherwise, outside the strictly vital portion of the bacillus, or it may be due to some fat, wax, or other covering material preventing this substance from being utilized by Johne's bacillus. In the light of some recent experiments the latter possibility seems improbable, as we have been unable to extract any substance suitable for the growth of Johne's bacillus. These experiments are being continued.

"While in this paper we cannot enter into the controversy concerning the relationship between the human and bovine types of tubercle bacilli, yet, incidentally, we venture to remark that, in spite of all that has been written in this country, we are not yet convinced that the human and bovine types are only slightly different varieties of one and the same micro-organism. In this connection the difference between the two bacilli described above may be worthy of note and further investigation;"

In the same paper we wrote:

"We have not tested many strains of the bovine bacillus, and it is possible that Johne's bacillus will grow on some bovine strains, or on those strains which have been described as occupying an intermediate position between the typical human and typical bovine bacilli."

In describing the results obtained by M'Fadyean, Sheather, and Edwards, and the results obtained by

ourselves, we have thought it advisable to quote at some length in order that the reader may fully appreciate our respective opinions.

Nature of the "Essential Substance."—Since the publication of our experiments with media containing acid-fast bacilli, or extracts obtained from the bacilli, we have attempted to isolate the substance—the "essential substance"—contained in these bacilli and extracts, in the hope that we might be able to throw some light on its chemical nature. To carry out our experiments we obtained large quantities of *B. phlei* by growing the bacillus on glycerine-peptone-beef broth for fourteen days at 37° C.; the cultures were then filtered, and the growth washed and dried. We prepared the alcoholic extract as before, and from this we attempted by means of various solvents to separate the "essential substance." In the first place, the wax was obtained in a fairly pure state by repeatedly dissolving in hot alcohol, precipitating by cooling, and filtering. The wax, when added to media, gave negative results, and need not be considered further.

The two portions of extract soluble in cold alcohol, when evaporated to dryness, were extracted with such solvents as ether, petroleum ether, acetone, and methyl alcohol, but with very unsatisfactory results, the essential substance being mixed up with most of the portions obtained. The experiments took a considerable time to carry out, as the only way in which we could test for the substance was by preparing media containing the separate portions, and by testing the growth of Johne's bacillus on the various batches of media. Usually it was necessary to incubate the cultures for at least two months before we could be certain that no growth of Johne's bacillus had

occurred, and, as we have already remarked, in point of fact some growth took place on most of the media.

The portion that was insoluble in chloroform, but soluble in water, always gave a good growth, and we were able to reduce the amount of essential substance in the chloroform soluble portion by extracting with water, especially by dissolving it in a little chloroform and shaking with water in a separating funnel.

In the case of the bovine tubercle bacillus grown on Dorset's egg medium, we obtained apparently the same extracts as with the *B. phlei*, yet Johne's bacillus failed to grow on media containing the extracts of the bovine tubercle bacillus.

To investigate the nature of the "essential substance," we now carried out experiments to ascertain, if possible, the general nature of the substance or substances from which it was formed, and from these experiments we soon obtained some interesting results. We experimented with *B. phlei* grown on various media, and found that when grown on glycerine-agar, or glycerine-broth, the bacilli formed a good medium; but, on the other hand, when grown on Dorset's egg medium, the results were not nearly so good, and when grown on glycerine-liver broth for four weeks at 37° C., the bacillus was useless for making media for Johne's bacillus. From *B. phlei* grown on liver-broth we obtained apparently the same extracts as before, but the extracts were unsuitable for making into media. The same results were obtained, as a rule, with the other acid-fast bacilli. The human and avian tubercle bacilli made the best media for Johne's bacillus when they had been grown on glycerine-beef broth, or glycerine-beef broth-agar, although the avian type,

even in this case, was not so good as the human type. We also obtained some positive results with a bovine strain grown on glycerine-beef broth, but here again the results were not nearly so good as with *B. phlei.*

A similar experiment was carried out with a freshly isolated strain of Johne's bacillus grown on the timothy-grass bacillus-egg medium, and with a strain of Johne's bacillus acclimatized to grow without the essential substance and grown on ordinary glycerine-peptone-beef broth. In this experiment a freshly isolated strain of Johne's bacillus grew on an egg medium in which was incorporated 1 per cent. of the dead acclimatized Johne bacilli which had been grown on the glycerine-beef broth; but, on the other hand, it failed to grow on an egg medium in which were incorporated the dead bodies of a freshly isolated Johne bacillus which had been grown on an egg medium containing 1 per cent. of dead *B. phlei.* Thus we obtained the same result with Johne's bacillus as with the bovine tubercle bacillus. This experiment shows also that Johne's bacillus has not become acclimatized to grow without the essential substance, but that it has acquired the power of forming it from the medium; and it is interesting to note that the strain of Johne's bacillus that has become acclimatized to grow on glycerine-beef broth still gives no growth on ordinary Dorset's egg medium. So presumably it cannot form the essential substance from that medium, although, of course, like an unacclimatized strain of Johne's bacillus, it will grow well on the egg medium if it contains some essential substance obtained from some other acid-fast bacillus, including the same acclimatized strain of Johne's bacillus grown on ordinary glycerine-beef broth.

The fact that our strain of the bovine tubercle bacillus, when grown on Dorset's egg medium, appears to contain no essential substance does not prove that no essential substance is formed, but that no reserve is formed that can be extracted; in other words, that only just sufficient is formed for the immediate requirements of the bacillus; and the same is probably true in the case of *B. phlei* grown on glycerine-liver broth. Therefore in most of these experiments that we have classed as negative a small quantity of the essential substance may have been formed for the immediate use of the bacillus.

Our experiments also showed that certain strains of the human tubercle bacillus seemed to form but little reserve essential substance when first isolated from the body on Dorset's egg medium; and we thought that the difficulty experienced in isolating this bacillus from certain body fluids, such as pleural fluids, even though their presence can be demonstrated by the inoculation of a guinea-pig, may be due to a temporary inability of the bacillus to form the essential substance. To test this we tried to isolate the bacillus from these fluids on media containing the essential substance, and we tested tubercular pus from cows in the same way. Our experiments were limited in number, but they certainly indicated that a small quantity of the essential substance (or of bacilli containing the essential substance) improves a medium used for isolating these bacilli.

On reviewing the experiments so far carried out, it is obvious not only that the essential substance is soluble in water and present in the alcoholic extract of *B. phlei*, only in a very small quantity, but also that the medium on which the bacillus is grown plays an important part in determining the production or

otherwise of the essential substance, and the same rule holds good for other acid-fast bacilli, including the bovine type of tubercle bacillus.

Reasoning now on the same lines that first induced us to try the addition of other acid-fast bacilli to a medium for growing Johne's bacillus, we thought that as the essential substance was necessary for the growth of Johne's bacillus, so, too, the material from which such organisms as the *B. phlei* formed the essential substance, would be more or less necessary for the growth of *B. phlei*, for the simple reason that the essential substance, or an allied substance, or one of a number of allied substances, must be formed by *B. phlei* before that micro-organism can grow.

Now, it is well known that fatty substances play an important part in the metabolism of the acid-fast group of bacilli, and also that certain alcohols, such as glycerine and mannite, greatly improve the media on which the bacilli can be grown. These and allied substances, then, were the first to receive attention. They were added to ordinary peptone-beef broth, a number of other fluid media being prepared as controls. On each of the media *B. phlei* was tested, not only as regards growth, but also as regards the formation of the essential substance. The following are the most important of the media that were tested:

Naegeli's fluid + 4 per cent. glycerine.
Naegeli's fluid + 4 per cent. glycerine + 1 per cent. peptone.
Watery extract of hay.
Watery extract of carrot.
Gordon's lemco medium.
Gordon's lemco medium + 4 per cent. glycerine.
Watery extract of fish + 1 per cent. peptone.
Watery extract of brain + 1 per cent. peptone.
Watery extract of mammary gland of cow + 1 per cent. peptone.
Watery extract of beef + 1 per cent. peptone.

Besides these we tested an extract of beef, to portions of which were added one of the following substances in the following quantities:

	Per Cent.		Per Cent.
Glycerine	1	Tertiary butyl alcohol ...	1
Glycerine	2	Glucose	1
Glycerine	4	Dextrose	1
Glycerine	10	Lævulose	1
Mannite	1	Lactose	1
Mannite	2	Maltose	1
Erythrite	1	Saccharose	1
Erythrite	2	Rhamnose	1
Dulcite	1	Galactose	1
Methyl alcohol	2	Raffinose	1
Methyl alcohol	5	Potassium sodium tartrate	1
Absolute alcohol	2	Amygdalin	1
Absolute alcohol	5	Asparagin	1
Propyl alcohol	1	Glycol	2
Isopropyl alcohol	1		

To all, except the Naegeli media, ½ per cent. of sodium chloride was also added.

B. phlei was grown on flasks of all the media, many of which were also used for growing the "nasenschleim" bacillus and Grassberger's bacillus. The quantity of growth obtained with *B. phlei* varied considerably, the best being on the broth containing glycerine; but growth was also fairly good on the media containing mannite, erythrite, glucose, dextrose, lævulose, 2 per cent. absolute alcohol, or 2 per cent. propyl alcohol, and also on Gordon's fluid+glycerine and on the hay extract. In the case of the "nasenschleim" bacillus and Grassberger's bacillus much the same results were obtained, and in every case the bacilli had formed the essential substance—that is, when killed and dried and added to egg, they made good media for Johne's bacillus. The media, however, varied, and although space will not allow us to give details of the many hundreds of experiments carried

out, we may say that from the results we were inclined to believe that the essential substance was formed from such substances as glycerine, mannite, and absolute alcohol.

If such be the case, the next problem was to find out how these substances might be changed by *B. phlei* in the process of forming the essential substance for its metabolism. We know that in the process of fermentation hydration and oxidation take place. Even in the case of alcoholic fermentation, in the late stages of the process, when the yeast is growing as a film on the surface of the fluid, the alcohol may become oxidized and almost entirely disappear, and the probability is that either outside the cell or inside the cell some organic acid is one of the final products formed for the *benefit* of the bacterial or yeast metabolism, and it may be taken as certain that this acid varies in the case of different bacilli. It may be, in fact, that the carboxyl group (COOH) has to be formed in certain compounds before such micro-organisms can obtain their carbon from sugars and alcohols.

In the case of *B. coli*, the large quantity of acid produced in media containing sugars may be the same process, the conditions being such that more acid is produced than is necessary or beneficial, a phenomenon of over-production so often met with in lowly organized life.

If, as we believe, some acid is necessary, then it is quite clear that *B. phlei* and allied bacilli must contain a special oxidizing enzyme, in order that they may produce from such substances as glycerine the particular acid which is necessary for each bacillus.

Now, from our experiments we were led to believe that the essential substance might be an organic acid, and that the reason why it is necessary to add this

substance to egg before any growth of Johne's bacillus can be obtained is that Johne's bacillus has lost its power of producing the necessary oxidizing enzyme, such an enzyme being unnecessary in the animal body, where the special acid (or acids) is produced by the ordinary cell metabolism of the host. It is not to be presumed that the acid is formed for the benefit of the bacterium, but that the acid being formed by the host, the bacterium, when in remote ages it commenced to become pathogenic, either found it suitable for its own metabolism, or it mutated, so that it could utilize the special acid present in the host. In either case the bacillus would no longer require an enzyme to produce the acid, and as it became more strictly pathogenic, it would no doubt cease to be capable of forming both the enzyme and the acid. We know that bacteria do vary, or mutate, in this manner; for instance, in the typhoid-coli group the most pathogenic members, compared with the non-pathogenic varieties, have very largely, though not completely, lost their power of producing acids. On the other hand, one of us (F. W. T.) has induced the typhoid bacillus to acquire the power of producing acid from lactose; this was done by subculturing the bacilli for a long period of time in a poor medium, containing lactose and no other sugar, thus forcing the bacilli either to utilize the lactose, or hunger strike and perish. Now, the special acid in the animal body may not be a *waste* product of the cell, but may be essential to the host, although it may only be present in the tissues in an exceedingly small quantity. This, if true, might account for the long incubation period in such conditions as Johne's disease and leprosy; for the small supply of acid would soon be used up by the bacilli, and there would be no more available until the host

reacted, so as to produce some more acid for its own use. But we know that in the process of repair there is usually over-production, a rule that is well illustrated in the formation of antitoxins; so that one would expect an over-production of the acid, and this, again, would be more favourable to the bacilli, or, if these had been killed, to a second infection of bacilli.

Although a great deal of what has been said is theoretical, it is not entirely so, as our experiments alone suggested that the essential substance might be an organic acid; so we again started to investigate the alcoholic extract of *B. phlei.* The extract obtained from 100 grammes of dried bacilli as a whole showed but slight acidity; but when separated into different portions with the solvents already mentioned, we found that some were markedly acid, and, further, that those portions that were most acid made the best media for Johne's bacillus. Using that portion of the extract which is insoluble in chloroform and soluble in water, we attempted to precipitate the acid with baryta water. Only a small precipitate formed, so the fluid was evaporated to dryness, and the sticky mass so obtained heated with methyl alcohol. Most of the mass dissolved, but a very small insoluble portion (about 0·007 gramme) remained. This was incorporated in 30 c.c. of egg medium, which was distributed in tubes, and sterilized as usual. A good result was obtained—that is, Johne's bacillus grew well on the medium, and no growth took place on a control medium made up at the same time with the same mixture of eggs. The test was repeated on several tubes of both media with another strain of Johne's bacillus, and the same result was obtained. Control media, containing small quantities of barium hydrate, gave negative results. This experiment is

interesting, not only because it supports our theory, but also because it shows what a small quantity of the essential substance is sufficient to stimulate the growth of Johne's bacillus.

More recently we have attempted to obtain a pure acid from the barium and sodium salts, but without success, the chief difficulty being to obtain a sufficient quantity of the barium salt. In view, however, of the positive results obtained with the barium compound, we now directed our attention to known organic acids.

In the first place it will be remembered that from our experiments with *B. phlei* we came to the conclusion that the essential substance might be formed by this bacillus from certain alcohols and sugars, so as substitutes for the essential substance we now tested acids that can be formed from these substances. The following is a list of acids investigated: Glycerine, tartronic, gluconic, saccharic, mucic, fumaric, malic, aconitic, glycolic, glyoxylic, succinic. Glyceric aldehyde and glycerose were also tested.

Each of these substances was made up into media, and as a basis the same glycerine-saline-egg mixture was used as in the previous experiments. Each acid was tested in percentages varying from 0·01 to 1·0, and in several series of media the acid was previously neutralized with sodium hydrate or baryta water. Tubes of each batch of medium were inoculated with Johne's bacillus, and after incubation for some weeks at 37° C., a slight growth was obtained on certain of the tubes containing glycerinic acid, and to a less extent on those containing tartronic acid, and on those containing gluconic acid.

In all these cases about 1 per cent. of acid was present, but it had been almost completely neutralized with NaOH before making into medium. We also

obtained slight growth on a medium that contained 0·2 per cent. of glycerose. The strain of Johne's bacillus had been grown on timothy-grass bacillus-egg medium for about twelve months, but still gave no indication of growth on ordinary Dorset's egg medium.

Although from the experiments it appears that not one of the acids enumerated above is identical with the essential substance, yet the results obtained with glycerinic, tartronic, and gluconic acids are interesting, and it is possible that the acid constituting the essential substance is allied to the acids mentioned. More recently we have tested other organic acids, including various tartaric, oxalic, and phosphoric acids. We have also tested acids that contain other elements—chlorine, nitrogen, or sulphur—in combination; but all the results were negative.

Although we have been unable to discover an efficient substitute for the essential substance, our experiments, considered as a whole, certainly suggest that it is some organic acid. However, whatever its nature may be, it will probably be difficult to obtain sufficient material to determine its constitutional formula, although in the future it may be found possible to discover the nature of the constituent elements, and the structure of the essential organic radicle in the molecule.

The relationship of Johne's bacillus to the tubercle bacilli and other acid-fast bacilli, and the possibility that the essential substance may influence the pathogenicity of Johne's bacillus, are discussed more fully at the end of Chapter IX.

In the light of future research, some of the theories we have put forward may prove to be wrong; but we have thought it advisable to give them, not only that

the reader may be able to follow the reasoning which led us from one series of experiments to the next, but also because we believe that the same fundamental laws will be found to apply to Johne's bacillus and to the lepra and tubercle bacilli. We hope, too, that the experiments that we have carried out may help to throw some light on the vital chemical changes that occur in the life-history of these bacilli, both when outside and when inside the animal body.

Description of Cultures.—In giving the experiments on the cultivation of Johne's bacillus, we have frequently described the cultures obtained, but in most cases the description refers only to young primary growths, so that a more complete description of vigorous growing and older cultures is not out of place. Details of the preparation of the media have already been given.

Egg Media containing the Essential Substance.—These media, as we have seen, may be made with tubercle or other acid-fast bacilli that contain the essential substance, or with the essential substance extracted from these bacilli; they must contain about 4 per cent. by volume of glycerine. On such media Johne's bacillus in primary cultures grows as tiny, dull white colonies, which are rarely visible to the naked eye in less than four weeks. The colonies are irregularly round, and either remain quite small and discrete, or they may coalesce. If only a few are present, they increase in size, and when older become more elevated, especially in the centre, and turn dull yellowish-white in colour. The edges of the colonies remain thin, and from the margins numerous irregular elevations gradually rise towards the centre, and end in a peak, which is sometimes very markedly elevated. The elevations are most marked on dry media and on those

in which the essential substance is not mixed well with the egg. These elevated colonies are well shown in Plate IX., Figs. 1 and 2. When streak subcultures are made on to the same media with a platinum loop, growth occurs along the needle track. The growth in this case does not greatly differ from that obtained in primary cultures, except that it is more rapid, and the colonies usually coalesce to form a continuous irregularly heaped-up growth. This is shown on a tube of medium that contains the chloroform insoluble portion of a cooled and filtered alcoholic extract of *B. phlei*, and is illustrated in Plate VI., Fig. 1. If, in place of the saline in the medium one uses peptone-beef broth, the growth is certainly more vigorous; this is illustrated in Plate IX., Fig. 3. If the broth is made from brain, such as sheep's brain, the improvement is still more marked (see Plate VII., Fig. 1). In this case the growth cannot be distinguished from that of a vigorous-growing culture of the human tubercle bacillus on ordinary Dorset's egg medium (see Plate VII., Fig. 2), and is more copious than that of either the bovine or the avian tubercle bacillus grown on Dorset's egg medium. A culture of the avian type of bacillus grown on egg medium is shown in Plate VII., Fig. 3. If, in these media, the egg is replaced by blood-serum or ascitic fluid, Johne's bacillus does not grow so well.

Agar Media containing the Essential Substance. — When the essential substance or a bacillus containing the essential substance is added to glycerine-peptone-beef broth-agar, the growth of Johne's bacillus is slower and not so vigorous. The growth, however, does not greatly differ from that which occurs on the egg media, except that it is usually more coloured. The agar medium, however, is improved by the addition of blood-serum, or ascitic or pleuritic fluid, and growths

PLATE VII.

FIG. 1. FIG. 2. FIG. 3.

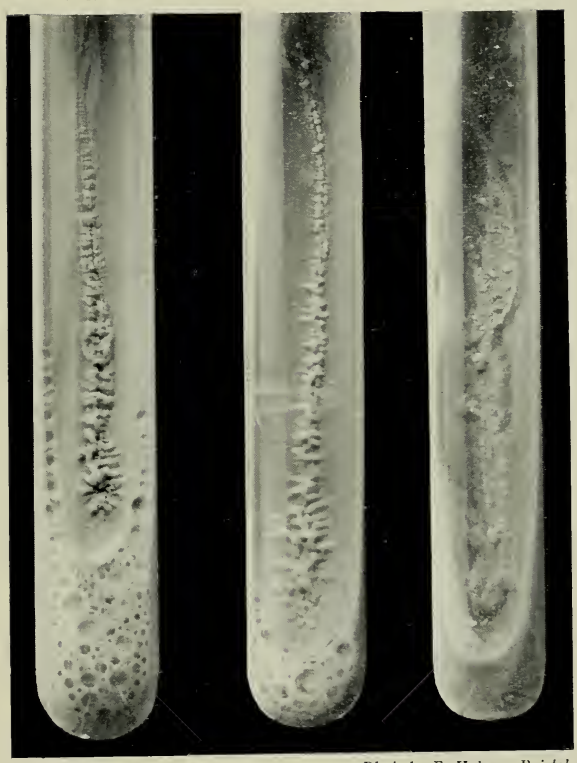

Photo by F. Holmes, Bristol.

CULTURES OF JOHNE'S BACILLUS.

FIG. 1.—Streak culture of Johne's bacillus on an egg medium made up with sheep's brain broth containing peptone and glycerine, and containing 1 per cent. of dead *B. phlei.*

FIG. 2.—Streak culture of the human tubercle bacillus on Dorset's egg medium.

FIG. 3.—Streak culture of the avian tubercle bacillus on Dorset's egg medium.

[*To face page* 104.

on two tubes of one such medium, which contains dog's unheated ascitic fluid which had been passed through a sterile Doulton white filter, are shown in Plate VI., Figs. 2 and 3.

Fluid Nutrient Media.—Ordinary peptone-beef broth containing glycerine and the essential substance, or a bacillus containing the essential substance, does not form a good medium for a freshly isolated strain of Johne's bacillus. There is great difficulty in inducing the bacillus to grow on the surface of the medium. After three or four weeks, growth occurs at the bottom of the flask as tiny yellowish-white grains, and these gradually increase in size and number. It is usually some months before any growth takes place on the surface of the medium, and when it first appears it occurs as a very delicate film. In subcultures the growth becomes thicker and more vigorous, although in this case it rarely covers the whole surface of the medium. After subculturing several times, surface growth may occur as very thick, knobby, and irregular masses. We found this knobby condition particularly well marked with a strain of the bacillus which, after two years, had become acclimatized to grow on glycerine-beef broth without the essential substance; such a growth is shown in Plate VIII. The growth extends from these knobby masses by forming fresh rings of thin growth, which in turn also become heaped up and knobby, or in the same way entirely fresh areas may form. Several of the thin patches may be seen very faintly in the plate. As the bacillus becomes better acclimatized to the glycerine-peptone-beef broth medium, the growth ceases to be characteristically knobby, and becomes more like that of the tubercle bacilli.

CHAPTER VII

VACCINES: DIAGNOSTIC AND CURATIVE

The premature announcement in Germany, in 1891, of the discovery by Koch of tuberculin and its curative properties was followed by the administration of excessive doses. This caused a temporary discredit to fall on the use of the reagent as a curative vaccine for phthisis in man, and to the present day opinion is still divided on this subject. But as a diagnostic reagent for cattle, tuberculin, in competent hands, has proved of the greatest value in eradicating tuberculosis from affected herds, and in preventing its reintroduction by newly-bought animals. Probably it was the success claimed for tuberculin which led the Russians, Kalning and Helman, to prepare mallein on the same lines. Independently of the reduction of the equine population owing to mechanical traction, the proportion of horses affected with glanders in Great Britain has been enormously reduced, and Hunting's work on this disease in London has probably been the means of preventing the infection of many men whose work brings them in contact with large numbers of horses.

The tuberculin test as applied to cattle is now familiar to all intelligent stock-owners. The preparation of the reagent varies in small details in different laboratories, but, broadly speaking, a glycerine-broth culture of the tubercle bacillus is killed by heat, filtered to

PLATE VIII.

Photo by F. E. Barnard, London.

CULTURE OF ACCLIMATIZED JOHNE'S BACILLUS ON ORDINARY GLYCERINE-PEPTONE-BEEF BROTH.

Note—Dense growth on surface.
,, Small granules of growth at bottom of fluid.
,, Very delicate surface growth in several places.

[*To face page* 106.

remove the bacilli, and the filtrate evaporated to one-tenth its original volume. Before it is sold to be used by veterinary surgeons it is diluted with nine times its volume of a weak solution of carbolic acid in 10 per cent. glycerine. The dose of the diluted vaccine for an adult bovine is usually about 3 to 4 c.c. when given as a subcutaneous injection under the skin of the neck or behind the shoulder. If the vaccine is prepared so that its strength differs from that given above, then the dose will vary accordingly. The temperature of the animal to be tested is taken for one or two days, and if found to be within normal limits, the injection is then given. The temperature is noted at the time of injection, and also at the ninth, twelfth, fifteenth, and eighteenth hours following the inoculation. In an animal in which the temperature at the time of inoculation was not above 103° F., a rise of temperature to 104° F. or over is considered as a positive reaction—*i.e.*, the animal is tubercular. If the initial temperature is above 103° F., the test should be postponed for a few days. With this test the temperature frequently rises to 105° or 106° F., and in such cases the animal shows signs of fever, loss of appetite, etc. In experienced hands the proportion of errors is exceedingly small, although in very advanced cases a negative result is not uncommonly obtained.

There are several modifications of the tuberculin test.

The ophthalmic test is performed either with Calmette's tuberculin or with a 1 per cent. solution of Koch's Old Tuberculin. Calmette's preparation consists of an alcoholic precipipate of tuberculin to which no preservative has been added. Wolff-Eisner gives the following description of the method of preparation: A glycerine-broth culture of the bovine tubercle bacillus,

six weeks old, is autoclaved at 110° C. for twenty minutes, and then evaporated at 80° to 90° C. to a tenth of its volume. The fluid is now filtered and precipitated with 95 per cent. alcohol; the precipitation is hastened by the addition of a small quantity of sodium chloride. The fluid is now passed through filter-paper and the precipitate scraped off and dried *in vacuo*. The precipitate can be purified by repeating the process. For use, the white precipitate is made up into a 1 per cent. solution with sterile water.

To carry out the test, a few drops are placed into one of the eyes of the animal, and the other eye is used as a control. The presence or absence of a reaction is judged by the amount of conjunctival inflammation produced in the treated eye. In a tubercular animal this reaction may be well marked, and a mucopurulent secretion may result for a few days. The ophthalmic or eye test has been used for the detection of tuberculosis in animals that have been inoculated by fraudulent dealers with large doses of tuberculin just before sale in order to prevent any reaction if tested shortly afterwards in the ordinary way. The conjunctival reaction is not prevented by a previous subcutaneous inoculation of tuberculin.

Intradermal injections of tuberculin, which cause an inflammation or thickening of the skin in tubercular animals, have also been used, and a tuberculin prepared with a non-irritant lanoline base may be given as an inunction into sound or slightly scarified skin. These methods, however, are not of practical value on a large scale, having no advantages as primary tests over the simple subcutaneous injection.

It is often not necessary to use an autogenous vaccine to produce a reaction. Man, suffering from certain forms of leprosy, reacts to a human tuberculin if suffi-

cient is given. On the other hand, it must be remembered that vaccines prepared from almost any acid-fast bacillus will produce a reaction in such conditions as tuberculosis, leprosy, and Johne's disease, provided that a sufficiently large dose is given; but with such a dose animals suffering from other diseases, and normal animals are very liable to react, and from this it follows that any reaction obtained in the test animal is worthless or even misleading.

To obtain a specific and reliable diagnostic vaccine, it should be prepared from the same species of bacillus that causes the particular disease, in which case it is necessary to give only a relatively small dose to produce a reaction, in fact, a dose sufficiently small to avoid a definite reaction in animals suffering from allied diseases or by normal animals. Other factors remaining constant, it may be said in general that the closer the relationship between the specific variety of bacillus causing the disease and the bacillus from which the vaccine is prepared, the more reliable and specific the test becomes.

In the case of Johne's disease the absence of a pure culture of Johne's bacillus led several workers to investigate vaccines prepared from allied acid-fast bacilli. O. Bang, in 1907, tested cattle suffering from pseudo-tuberculous enteritis with a vaccine made from *B. phlei*, and the present writers have since tested a strain of *B. phlei* in the same way, both on naturally infected and on artificially inoculated animals.

In 1907-08 O. Bang tested cattle suffering from Johne's disease with a tuberculin prepared from tubercle bacilli isolated from birds. The vaccine used was prepared from a four months' old glycerine-broth culture. The filtrate obtained from this culture was evaporated to one-tenth the original volume, and one

part of this concentrated liquor was added to four parts of a ½ per cent. aqueous solution of pure carbolic acid. Of this reagent Bang gave the following doses:

Animals over two years old	...	10 c.c. subcutaneously.
Animals one to two years old	...	7–8 c.c. ,,
Animals six months to one year old		5–6 c.c. ,,

It should be noted that the dose given (10 c.c. of a 1 : 4 dilution) is about six times the dose of tuberculin used (3-4 c.c. of a 1 : 9 dilution) when testing an adult animal for tuberculosis.

O. Bang found that in very advanced cases of the disease a good reaction was not obtained with avian tuberculin. In his report he gives the results of about fifty tests, from which we have selected seven of the most marked reactions. The temperatures are given in degrees Centigrade in the following table:

Number of Case.	At Injection.	8th to 9th Hour.	11th Hour.	13th Hour.	15th Hour.	17th Hour.	19th to 20th Hour.
4	38·2	40·4	40·9	40·6	40·7	41·0	41·0
5	38·7	38·5	39·1	39·6	40·2	40·6	39·4
8	38·7	40·2	40·4	40·4	41·1	40·1	39·8
9	38·6	40·6	41·2	40·0	40·3	40·4	39·6
17	38·8	40·0	41·1	40·7	40·9	39·5	39·1
26	38·0	39·7	40·7	40·8	40·4	39·9	
28	38·0	38·8	38·6	39·9	40·1	40·8	40·2

If the temperatures detailed above were the result of tests with ordinary diagnostic tuberculin in tubercular cattle, slaughter of all animals would be justified, as in every case the maximum exceeds 105° F. (40·6° C.), and in some cases it reaches 106° F. (41·1° C.).

With the same vaccine O. Bang tested about 1,700 animals, mostly herds of tuberculosis-free Jersey cattle, and he found that from 6 to 35 per cent. of adult cattle reacted. Of those that reacted 34 animals (tuber-

culosis-free) were tested again with avian tuberculin eight months after the first test, and only 88 per cent. gave a positive result. This may have been due to spontaneous recovery in the interval between the tests, or to a curative effect of the vaccine; or, again, it may have been due to failure on the part of the vaccine. Other observers have found that an animal may give quite different results within a month with the same dose of avian tuberculin (*vide infra*).

O. Bang recommends mixing avian tuberculin with ordinary diagnostic tuberculin with the object of testing for Johne's disease and tuberculosis at the same time. He concludes that if an animal reacts to avian tuberculin it may safely be assumed to be suffering from pseudo-tuberculous enteritis, but a negative result would seem to be of doubtful value.

In 1909, Le Sueur of Jersey, in conjunction with O. Bang, tested 194 head of cattle with avian tuberculin, and in a private communication Le Sueur writes: "With regard to the avian tuberculin test I do not regard it as practical, and feel convinced that the reaction which takes place after injection is simply due to the large amount of tuberculin injected." In the same year Malm tested with avian tuberculin two calves in which the disease had been produced by feeding with infected intestine. The first animal was tested subcutaneously on five occasions, with two positive and three negative results. In the tests that proved positive large doses were given. The ophthalmic test was applied with avian tuberculin three times, two reactions being positive and one uncertain. Of intradermal tests one was positive and two were negative. The animal was slaughtered, and the diagnosis of Johne's disease confirmed by B. Bang. The second calf, with avian tuberculin, gave three

positive and two negative reactions. When killed it showed the characteristic lesions of the disease.

In his paper (*Norsk Veterinær-Tidsskrift*, No. 8, August, 1911), Malm gives the following notes on the two calves:

CALF A.

3.4.08.	Calf fourteen days old, fed with infected intestine in milk.
14.5.08.	Again fed from another animal. No fever, no diarrhœa, no bacilli in fæces till 23.6.09.
10.3.09.	2 c.c. avian tuberculin ; well-marked reaction—38·8 to 40·9. No diarrhœa, no fever next day.
2.4.09.	½ gramme Old Tuberculin ; negative reaction.
5.4.09.	Uncertain reaction (ophthalmic) with avian tuberculin.
13.4.09.	Good ophthalmic reaction in other eye.
23.4.09.	1 c.c. fish tuberculin + 1 c.c. blindworm tuberculin subcutaneously ; negative reaction.
17.6.09.	Intradermal reaction, avian tuberculin, positive.
23.6.09.	Bacilli in fæces for first time.
25.6.09.	Simultaneous, intradermal, and ophthalmic tests with avian tuberculin. Ophthalmic test, positive.
28.6.09.	Intradermal test, negative.
7.7.09.	Diarrhœa noticed first time. 0·35 gramme avian tuberculin ; negative reaction.
28.9.09.	Avian tuberculin ; negative reaction.
19.11.09.	Diarrhœa again noticed.
13.12.09.	1 gramme avian tuberculin ; negative reaction.
20.1.10.	Large number of acid-fast bacilli in fæces.
29.1.10.	2 grammes avian tuberculin ; good reaction (38·5 to 40·2).
31.1.10.	Killed. All the characteristics of a typical case of Johne's disease. Diagnosis confirmed by B. Bang, then in Christiania.

Scrapings of bowel were inoculated into three rabbits and eight guinea-pigs with negative results.

CALF B.

16.4.10.	Fourteen days old. Fed with 150 grammes mucosa of bowel of Case 13 in warm milk.
21.6.10.	Positive reaction to ½ gramme of avian tuberculin (38·9 to 40·1). Marked diarrhœa after reaction, and many acid-fast bacilli in fæces.
8.8.10.	Old tuberculin ; negative reaction.

27.9.10. ½ gramme avian tuberculin ; positive reaction (39·1 to 40·1).
6.1.11. Same dose of Old Tuberculin ; negative.
20.1.11. Reacted with avian tuberculin (40·1).
1.4.11. No acid-fast bacilli in fæces.
4.4.11. 1 gramme of avian tuberculin gave a negative reaction.
10.4.11. 1 gramme Old Tuberculin ; negative.
18.4.11. 1 gramme Old Tuberculin ; negative.
10.5.11. Killed. Proved to be a typical case of Johne's disease.

Miessner and Trapp, using avian tuberculin received from Bang, tested a series of experimentally inoculated animals. Their results are given in the table on p. 114.

Here the first five calves certainly "reacted," and suffered an attack of diarrhœa as a result of the disturbance.

G. P. Male has used avian tuberculin as a test for this condition, the reagent being supplied to him by Stockman. The following are his results : Herd 1—Jersey cattle. One bull, ten cows, and eight heifers and calves were tested, and two cows and two heifers gave the following reactions :

Animal.	Temperatures (Fahrenheit Scale).				Post Mortem.
	Time of Injection.	9th Hour.	12th Hour.	15th Hour.	
(*a*)	100·4	104·0	103·2	101·0	+ Johne's disease
(*b*)	101·0	102·0	104·0	101·4	+ Johne's disease
(*c*)	101·0	102·0	104·0	105·0	+ Johne's disease
(*d*)	101·4	101·4	103·2	99·2	+ Johne's disease

Case (*d*) was examined by Stockman post mortem, who gave it as his opinion that the animal was affected with Johne's disease, though no acid-fast bacilli were found. None of the animals showed any evidence of tuberculosis post mortem. Herd 2 : Thirty-two

Animal.	Date of Infection.	Infected with.	Method of Injection.	Temperature before Injection, Centigrade Scale.	Highest Temperature reached, Centigrade Scale.	Hour Maximum reached.	Other Effects.
Calf 62	16.2.09	Intestinal mucosa (Cow 6)	Per os	38·9	41·5	36	Diarrhœa
Calf 63	16.2.09	Mesenteric gland (Cow 6)	Per os	39·0	41·1	48	Diarrhœa
Calf 72	28.4.09	Mesenteric gland (Calf 182)	Subcutaneous	39·2	40·5	36	Slight diarrhœa
Calf 73	28.4.09	Mesenteric gland (Calf 182)	Subcutaneous	39·0	40·3	8	Slight diarrhœa
Calf 74	28.4.09	Intestinal mucosa (Calf 182)	Per os	39·1	40·1	8	Severe diarrhœa
Calf 122	3.9.09	Intestinal mucosa (Cow 9)	Per os	39·1	39·3	36	None
Calf 104	(?)	From mother	—	38·7	38·8	12	—
Calf 200	—	Control	—	39·1	39·4	10	—
Goat 105	3.7.09	Intestinal mucosa (Cow 8)	Per os	40·0	40·8	16	—
Goat 106	3.7.09	Intestinal mucosa (Cow 8)	Per os	39·1	39·6	10	—
Dog 76	28.4.09	Intestinal mucosa (Calf 182)	Per os	39·0	39·5	10	—
Dog 107	3.7.09	Intestinal mucosa (Cow 8)	Per os	39·5	39·7	8	—
Dog 108	3.7.09	Intestinal mucosa (Cow 8)	Per os	39·5	39·6	8	—
Dog 250	—	Control	—	39·8	39·9	14	—

Jerseys of various ages gave the following reactions:

Animal.	Temperatures (Fahrenheit Scale).			
Number.	Time of Injection.	9th Hour.	12th Hour.	15th Hour.
5	101·2	101·0	103·8	103·8
8	101·0	103·2	101·4	101·0
20	101·0	104·0	102·4	102·2
24	101·8	101·8	103·2	104·0
29	101·0	101·0	105·0	102·0
Classed as doubtful:				
26	102·0	102·0	103·2	103·6

These cases were not confirmed by post-mortem examination, but were sent away at once from the farm. In the twelve months following the injections no other cases of Johne's disease occurred.

Two suspected cases on farms on which there was no history of Johne's disease were classed as negative from the following results:

Case Number.	Temperatures (Fahrenheit Scale).				
	Time of Injection.	9th Hour.	12th Hour.	15th Hour.	18th Hour.
1	101·0	101·4	102·0	101·2	101·0
2	100·2	102·0	101·2	101·0	101·0

Both these cases had been affected for some months with profuse diarrhœa, but on post-mortem examination they showed no characteristic corrugations as seen in Johne's disease, though the small intestines in both cases were inflamed and somewhat thickened: no acid-fast bacilli could be found in either case. Both

animals had given a negative result when tested with ordinary diagnostic tuberculin.

In only two of the nine cases detailed above, which Male classed as reactions, did the temperature rise above 104·0° F. Such reactions cannot be considered as very definite, and it is doubtful whether State legislation could be enforced and compulsory slaughter ordered, unless a more marked reaction can be produced. In the case of animals housed specially for the test and unused to being handled, the taking of the rectal temperature and the use of necessary restraint might lead to serious errors.

Since the publication of Male's results, the present writers have performed some tests with avian tuberculin. The vaccine in our hands gave negative results, and we concluded that, although some cases of Johne's disease undoubtedly react to large doses of avian tuberculin, the test is very uncertain, and is not of much practical value. Details of these experiments are given at the end of this chapter, where we discuss vaccines prepared from cultures of Johne's bacillus.

More recently M'Fadyean, Sheather, and Edwards have published results obtained with avian tuberculin. The test was applied to fourteen bovines, all of which were proved, on post-mortem examination, to be affected with pseudo-tuberculous enteritis. The dose of avian tuberculin given was 8 c.c.; it was given subcutaneously, and the results are shown in the table on p. 117, which we have compiled from the text of their paper.

In this table Cases XI. and XVI. may be considered positive on the first test, although the reaction in Case XI. is not very good. If one were testing with ordinary tuberculin for tuberculosis, Cases VI. and VIII. would be classed as doubtful.

Case No.	Temperatures in Degrees Fahrenheit.								Result.
	Time of Injection.	3rd Hour.	6th Hour.	9th Hour.	12th Hour.	13th Hour.	15th Hour.	21st Hour or Later.	
I.	101·8	...	102·8	103·4	102·0	...	102·8	...	−
III.	101·6	...	101·6	102·0	102·2	...	102·8	...	−
IV.	101·2	...	101·6	102·4	102·7	...	102·4	... 27th Hour.	−
V.	100·0	101·2	102·4	100·4	101·0	...	102·4	101·4	−
VI.	102·0	103·0	103·6	103·0	103·2	...	103·0	101·0	−(?)
VII.	101·0	102·0	102·0	100·8	102·2	...	101·4	101·0	−
VIII.	101·2	103·0	103·8	101·2	103·4	...	103·0	101·4	−(?)
XI.	101·2	102·2	103·6	102·2	103·0	...	104·0	101·0	+
XII.	101·2	102·0	101·8	103·0	102·4	...	102·0	...	−
XIII.	101·6	101·8	103·0	103·0	102·2	...	102·0	...	−
XIV.	101·6	101·8	103·2	102·8	102·2	...	102·0	... 24th Hour.	−
XVI.	101·6	...	102·2	101·2	104·6	106·4	103·6	103·2	+
XVII.	101·8	101·8	102·0	102·2	102·2	...	102·0	... 27th Hour.	−
XVIII.	102·5	101·4	101·4	101·6	102·1	...	102·0	102·1	−
Re-tested:									
III.	101·4	103·4	104·4	102·2	103·0	...	103·4	101·6	+
VII.	101·0	101·4	102·8	102·2	101·4	...	102·2	...	−
VIII.	101·4	101·4	103·0	105·8	103·4	...	102·6	...	+
XI.	102·6	102·0	104·0	105·4	105·2	...	105·0	...	+
XVI.	101·8	101·4	102·6	101·6	103·0	...	103·0	...	−

Retested, special dose:
Case IV. Time of injection, 102·6; eighth hour, 105·0; next morning, 104·2; evening, 103·6; following days varied from 100·0 to 104·0.

Case I. Left pleura and bronchial glands tubercular.
Case VII. Tubercular right pharyngeal gland on post-mortem examination.

Case III. was retested twelve weeks after the first test, and the figures given above "represent a fairly good general reaction produced rather early after the injection of the tuberculin."

Case IV. was retested after six weeks. A special vaccine was given: Fifty milligrammes of avian tubercle bacilli grown on the surface of glycerine-broth, and made into an emulsion with 2 c.c. of physiological salt solution, were injected into the jugular vein. "The temperature at the time of injection was 102·6° F., and by midnight it had risen to 105° F. On the following day the morning and afternoon temperatures were 104·2° and 103·6° F. respectively." This may be taken as a positive result, and the animal was afterwards found to be suffering from Johne's disease.

Case VIII. was retested after eight weeks. "A very good reaction was provoked on this occasion."

Case XI. was retested after the same lapse of time, and "a good general reaction was again produced."

Case XVI., which gave a marked reaction on the first test, on being retested after about nine weeks, gave what the authors considered was a "slight" reaction. Certainly the second set of figures in this case would not be sufficiently definite for a practitioner to risk the slaughter of a valuable animal. On post-mortem examination this case showed no decided macroscopic lesions, but gave "a decidedly positive result with regard to the presence of acid-fast bacilli."

Case XVII. had been previously tested by a veterinary surgeon with avian tuberculin, with a negative result.

We consider that the results obtained by these workers afford further proof that the avian tuberculin test is not a reliable means of diagnosing cases of Johne's disease, for in spite of the large doses of vaccine that were given, the majority of the cases failed to react, a result which is in agreement with that obtained by other investigators.

M'Fadyean, Sheather, and Edwards have also tried

ophthalmic, intradermic, and endermic tests on certain of the cases enumerated above.

The ophthalmic test with concentrated human tuberculin was applied with the object of discovering if any of the animals were tubercular. The reactions are described as follows:

Case III.: First test, "slight"; second test, "slight"; third test, negative.
Case V.: One test, "fairly distinct."
Case VI.: One test, "absolutely negative."
Case VII.: First test, "pretty good"; second test, "very well marked."
Case VIII.: First test, "extremely slight"; second test, "slight."
Case XI.: One test, "negative except for a trace of muco-pus."
Case XII.: One test, "fair."
Case XIII.: One test, "failed to provoke any."
Case XIV.: First test, "very feeble"; second test, "distinct."
Case XVI.: First test, negative; second test, negative; third test, negative.
Case XVII.: One test, "no indication."
Case XVIII.: One test, "failed to produce any."

The ophthalmic test with concentrated avian tuberculin gave the following reactions (the authors do not state whether the tuberculin used for Case VIII. was concentrated or not):

Case V.: One test, "very distinct."
Case VI.: One test, "no reaction whatever."
Case VII.: One test, "discharge of muco-pus."
Case VIII.: One test, "good reaction."
Case XI.: First test, "no indication whatever"; second test, negative.

Two intradermic tests were performed with concentrated avian tuberculin (Cases VII. and VIII.), and two tests with avian tuberculin the concentration of which is not definitely stated (Cases XI. and XVI.). The reactions were as follows:

Case VII.: One test, "only just an appreciable."
Case VIII.: One test, "quite negative."
Case XI.: One test, negative.
Case XVI.: One test, negative.

One intradermic test on Case III. with concentrated human tuberculin gave a "pronounced" reaction.

Two endermic tests with concentrated human tuberculin were negative (Cases XVI. and XVIII.).

The authors do not state the conclusions to be drawn from their experiments, and insufficient avian ophthalmic, intradermic, and endermic tests have been made to condemn these methods of diagnosis; but it seems improbable that these tests will be of more than scientific interest.

With regard to the avian tuberculin used as a subcutaneous test, the number of negative results may be due partly to the fact that the cases tested were somewhat advanced. O. Bang has found that very advanced cases do not react, and this, of course, is true with tubercular cattle inoculated with bovine tuberculin.

In many cases the temperature reactions obtained were not high, in spite of the large doses given—a result which is in agreement with what we have already stated in the beginning of this chapter with regard to heterogeneous vaccines. The results obtained by O. Bang were, however, the best that could be got in the absence of pure cultures of the bacillus.

In 1910 the present writers started experimenting on Johne's disease with the object of cultivating the causative bacillus and of preparing a reliable and specific diagnostic vaccine from the cultures obtained. After cultivating several strains of the bacillus on solid media, these strains were subcultured on to fluid media (*vide* Chapter VI.), and from these cultures vaccines were prepared.

Vaccine No. 1.—In the first experiments we used an alkaline peptone-bouillon containing 4 per cent. of glycerine and 1 per cent. of dried human tubercle bacilli. This was placed in Duclaux flasks, and steri-

lized by steaming. These flasks were inoculated with pure cultures of Johne's bacillus, and the main opening of each was capped with gutta-percha tissue. The flasks were incubated at 39° to 40° C. After the lapse of a month small, yellowish-white grains of growth became visible. These grew just above the sediment at the bottom of the flasks, and gradually increased in size and number. No film formation was observed. After two months the flasks were steamed, their contents passed through a Doulton white filter, and the filtrate so obtained placed in small sterile phials in quantities of 2½ and 5 c.c. The vaccine was not evaporated to obtain a more concentrated solution, as we considered this unnecessary for preliminary experiments.

Vaccine No. 2.—A second batch of vaccine was prepared in a manner exactly similar to the above, except that the dried human tubercle bacillus was replaced by the dried timothy-grass bacillus.

Vaccine No. 3.—A third batch was prepared by growing Johne's bacillus in a broth medium containing a glycerine-saline extract of the timothy-grass bacillus, the extract representing 1 per cent. of dried bacilli and 4 per cent. of glycerine. The medium used for preparing this vaccine was filtered and autoclaved before being inoculated, and in it the specific bacillus grew fairly well in tiny masses on the bottom of each flask.

Vaccine No. 4.—A fourth batch was prepared from the cultures of Johne's bacillus on the special timothy-grass bacillus-egg medium, the growth being scraped off and an emulsion made with Vaccine No. 3, described above.

Vaccine No. 5.—This was made in a similar manner to No. 4, except that the cultures of Johne's bacillus were suspended in 0·8 per cent. sodium chloride in place of Vaccine No. 3.

As controls to the above vaccines we used diagnostic

tuberculin prepared at the Pasteur Institute, diagnostic avian tuberculin obtained from the Royal Veterinary College, and a special timothy-grass bacillus vaccine prepared by ourselves. This last was made by growing the bacillus for about three weeks in a glycerine-broth medium, which was then steamed and filtered through a Doulton white porcelain filter. The sterile filtrate was placed in small sterile phials without previous concentration.

The results of tests conducted with these vaccines are summarized in tabular form on p. 123. Bovines Nos. 1 2, 4, and 5 are mentioned also in Chapter IX., where a full account of the pathological lesions present in each animal is given. Bovines 2, 4, and 5 are calves.

A + sign is used to indicate a positive reaction, and a — sign a negative one. On referring to the table, one will notice that Vaccine No. 1 gave a positive result with bovines Nos. 1, 2, and 5, a doubtful reaction with No. 4, and no reaction with the control bull. It is also seen from the tuberculin tests and from post-mortem examination that animals 1, 2, and 5 had contracted tuberculosis.

In considering the results, it must be remembered that Vaccine No. 1 was prepared by growing the specific bacillus on a medium containing the tubercle bacillus, and it might be expected that tubercular animals would react to the vaccine on account of the substances dissolved from the tubercle bacilli in the medium, in which case the positive results do not prove the presence of pseudo-tuberculous enteritis. That the rises of temperature with Vaccine No. 1 may have been caused by the presence of these substances is also indicated by the absence of any reaction in the control bull, and also by the negative results obtained with Vaccines Nos. 4 and 5, which contained no

Bovine No.	Date Inoculated.	Channel of Inoculation.	Vaccine 1. 27.6.11.	Tuberculin. 27.7.11.	Vaccine *B. phlei.* 28.8.11.	Avian Tuberculin. 6.9.11.	Vaccine 4. 12.10.11.	Vaccine 5. 18.10.11.	Date Killed.	Post-mortem *re* Tubercule.	Post-mortem *re* Johne's Disease.	Johne's Baccilli recovered in Culture.
1	19.11.10	Per os	5 c.c. +	3½ c.c. +	8 c.c. −	8 c.c. −	...	3 c.c. −	23.10.11	+	+	−
2	10.11.10	Intra-venous	2½ c.c. +	2 c.c. +	5 c.c. −	5 c.c. −	8 c.c. −	2½ c.c. −	24.10.11	+	−	−
4	21.2.11	Intra-peri-toneal	2½ c.c. ?+ ?−	2 c.c. −	5 c.c. −	5 c.c. −	8 c.c. −	...	16.10.11	−	−	−
5	21.2.11	Intra-venous	2½ c.c. +	...	...	...	...	...	12.7.11	+	+	+
Control calf No. 1 un-inoculated			2½ c.c. −	2 c.c. +	5 c.c. −	5 c.c. −	...	...	19.7.12	+	−	...
No. 2			...	2 c.c. −	5 c.c. −	5 c.c. −	...	...	19.7.12	+	−	...
No. 3			...	2 c.c. −	5 c.c. −	5 c.c. −	5 c.c. −	...	19.7.12	+	−	...
Bull, naturally contracted Johne's disease			1.9.11 10 c.c. −	...	...	1.11.11 8 c.c. −	10 c.c. −	...	17.10.12	+	+	+

tubercle bacilli. It may, however, be noted that the bull was a very advanced case, and that Vaccines Nos. 1 and 5 were weak.

From the results obtained with Vaccine No. 1, it is clear, as might have been expected, that to obtain a specific reagent for pseudo-tuberculous enteritis the tubercle bacillus must not be incorporated in the medium. It will be seen from the table that a timothy-grass bacillus vaccine caused no rise of temperature in normal animals or in animals suffering from tuberculosis or pseudo-tuberculous enteritis, although, being filtered through a porcelain filter, the vaccine was probably weakened. It will be seen also that Vaccines Nos. 4 and 5, which were prepared by growing Johne's bacillus in media containing the timothy-grass bacillus, caused no reaction in experimental or in control animals.

It may seem surprising that no reaction was obtained with Vaccines Nos. 4 and 5 in the animals affected with Johne's disease; but, in point of fact, the negative results might have been anticipated, as the greater part of the bacillary emulsion used was obtained from growths on solid media, the bacilli being made into an emulsion without any previous grinding. It must also be remembered that infected animals rarely, if ever, show a rise of temperature during the course of the disease, so that a more concentrated vaccine might be required for Johne's disease than for tuberculosis. If, as is held by many authors, the tuberculin reaction is an anaphylactic phenomenon, and is in no way related to the ordinary temperature changes found in a tuberculous animal, then the absence of a temperature in Johne's disease should be no guide to the quantity of specific vaccine likely to be necessary to produce a thermal reaction.

In the light of the results obtained with the vaccines described above, we now prepared a sixth batch.

Vaccine No. 6.—This was made by growing Johne's bacillus on ordinary glycerine-peptone-beef broth containing a glycerine-saline extract of *B. phlei* (the timothy-grass bacillus). This culture was grown for nine months at 39° C.; the whole was well shaken to form an emulsion of the bacilli, placed unfiltered into small phials, and heated for one hour at 62° C. The vaccine was first tested on three fully-grown bovines, and on five calves about seven months old, and was inoculated *intravenously* in doses varying, according to the size of the animal, from 5 to 10 c.c.

No. 1, a Jersey bull, No. 2, a shorthorn cow, and No. 3, a Jersey cow, all showed clinically the typical manifestations of advanced Johne's disease, which they had contracted naturally. Before we obtained these animals, they had been tested several times with diagnostic tuberculin with negative results, though the bull had been in our possession for over eighteen months, and had not been tested for tuberculosis during this period. Each animal received 10 c.c. of the special Vaccine No. 6. No. 1 gave a maximum temperature of 105° F., which was reached an hour after the inoculation. No. 2 gave a maximum temperature of 106·1° F. five hours after inoculation, and on the following day developed a violent diarrhœa, which persisted after the temperature had fallen. The fæces contained blood and mucus. No. 3 gave a maximum temperature of 104·8° F. This was reached in four hours, and the temperature was not taken again.

Bovine No. 1 developed symptoms of tuberculosis, so was killed (October 17, 1912). Post-mortem examination revealed typical Johne's disease in the intestine and mesenteric glands. The disease was in a very

Number.	Animal.	Definite Clinical Evidence of Johne's Disease with Diarrhœa.	Diagnostic Tuberculin.	Specific Vaccine of Johne's Bacillus. No. 6 Intravenous.	Specific Vaccine of Johne's Bacillus. No. 6 Subcutaneous.
1	Adult Jersey bull ...	+	−	+	...
2	Shorthorn cow	+	−	+	...
3	Jersey cow	+	−	+	...
	Experimental calf No. 1*a*	−	...	+	...
	Experimental calf No. 2*a*	−	...	−	...
	Experimental calf No. 3*a*	−	...	−	...
	Experimental calf No. 4*a*	−	...	+	...
	Experimental calf No. 5*a*	−	...	+	...
	Experimental goat No. 1	−	...	...	+
	Experimental goat No. 2	−	...	...	+
4	Jersey cow	+	−	...	...
5	Shorthorn cow	+	−	...	...
6	Shorthorn cow	+	−	...	...
7	Shorthorn cow	+	−	...	...
8	Shorthorn cow	−	−	...	...
9	Shorthorn cow	+	−	...	...
10	Shorthorn cow	+	−	...	...
11	Shorthorn cow	−	...	...	...
12	Shorthorn cow	−	...	...	...
13	Shorthorn cow	−	...	...	...
14	Shorthorn cow	−	...	...	...
15	Lincoln cow	−	...	...	...
16	Shorthorn cow	−	...	...	...
17	Shorthorn cow	−	...	...	...
18	Shorthorn cow	−	...	...	...
19	Shorthorn cow	−	...	...	...
20	Shorthorn cow	−	...	...	...
21	Shorthorn cow	...	...	...	...
22	Shorthorn cow	−	...	...	...
23	Shorthorn cow	+	...	...	...
24	Dutch cow	−	...	...	...
25	Shorthorn cow	+	...	...	...

Specific Vaccine of Johne's Bacillus. No 7 Subcutaneous.	Maximum Temperature after Vaccine of Johne's Bacillus.	Hour Maximum Temperature reached.	Diarrhœa started or increased after Vaccine.	Post-Mortem Evidence T. B.	Post-Mortem Evidence Johne's Disease.	Cultures of Johne's Bacillus obtained Post Mortem.	Number of Animal.
...	105·0	1	−	+	+	+	1
...	106·1	5	+	−	+	+	2
...	104·8	4	+	−	+	+	3
...	104·6	3	−	−	+	+	
...	102·6	5	−	−	+	+	
...	102·6	4	−	+	−	−	
...	105·0	6	−	+	+	+	
...	105·4?	24?	−	+	+	+	
...	106·6	9	+	−	+	+	
...	105·4	10	−	−	+	+	
+	104·6	6?	+	−	+	...	4
−	102·2	3	+	−	+	+	5
+	106·2	7	+	+	+	...	6
+	104·6	9	...	...	...	...	7
−	...	...	−	...	...	...	8
+	106·0	6	+	−	+	...	9
+	104·0	10	+	...	...	...	10
−	...	...	−	...	...	...	11
+	104·2	4	−	...	...	...	12
+	104·4	10	−	...	...	...	13
−	101·4	6	−	...	...	...	14
+	103·6	6	+	...	...	...	15
−	101·6	10	−	...	...	...	16
−	101·4	4	−	...	...	...	17
−	101·0	10	−	...	...	...	18
−	101·2	10	−	...	...	...	19
−	102·0	10	−	...	...	...	20
−	101·8	6	−	...	...	...	21
−	101·4	6	−	...	...	...	22
+	104·2	4	+	...	...	...	23
−	101·8	6	−	...	...	...	24
+	104·0	6	−	...	...	...	25

advanced stage, the bacilli being extremely numerous in the films and sections from the diseased gut. Tuberculosis, which must have been contracted while the animal was in our stables, was present in the lungs and bronchial glands.

Bovine No. 2 died three weeks after the inoculation (September 17, 1912), and on post-mortem examination showed the typical lesions of Johne's disease. The gut was very congested, and some hæmorrhages were present. There was no evidence of tuberculosis.

Bovine No. 3 died (August 26, 1912). Post-mortem examination showed advanced Johne's disease in the intestine and mesenteric glands, the bacilli being fairly numerous in films and sections from the diseased gut.

The five calves tested were those that are also mentioned in Chapter IX., and numbered 1*a*, 2*a*, 3*a*, 4*a*, and 5*a*, respectively. They had been inoculated with the strain of Johne's bacillus recovered from calf No. 5 (pp. 123 and 148). The animals did not thrive well, but they showed no clinical manifestations of Johne's disease. All were tested with Vaccine No. 6 about six months after inoculation with the living cultures; calves Nos. 2*a* and 3*a* each received 3 c.c., and calves Nos. 1*a*, 4*a*, and 5*a* each received 5 c.c. The following results were obtained: calves Nos. 2*a* and 3*a* showed no rise of temperature in six hours, and through an error were not tested again until twenty-four hours after the inoculation, when the temperatures were normal. The temperature of calf No. 1*a* rose to 104·6° F. in three hours, that of calf No. 4*a* to 105° F. in six hours, while calf No. 5*a* showed no rise in ten hours, but when taken the following morning—twenty-four hours after the inoculation—the temperature registered 105·4° F., and was dropping.

A few days after the vaccine tests the calves were

killed and post-mortem examinations performed. The results are summarized in the table, and a detailed description of the pathological lesions present is given in Chapter IX., pp. 150 and 151.

We now tested the vaccine by means of subcutaneous inoculations, and as at the time we had no more bovines at our disposal, we used goats. The goats tested were two which we had inoculated in July, 1911, with living cultures of Johne's bacillus. These animals are also mentioned (p. 151).

At the time of testing the vaccine, eleven months after the intravenous inoculation of the living culture, goat No. 1 was thin, but otherwise there was no manifestation of the disease. Goat No. 2, which had been inoculated into the peritoneal cavity, was apparently quite healthy. Each animal received 3 c.c. of the vaccine subcutaneously. The temperature of goat No. 1 rose to 106·4° F. in five hours, reached 106·6° F., and remained at 106° F. or over for fully eight hours; the rise of temperature was accompanied by some diarrhœa. The temperature of goat No. 2 reached a maximum of 105·4° F. at the tenth hour, but no diarrhœa ensued. Both animals were killed and post-mortem examinations made. Goat No. 1 showed Johne's disease throughout the intestines and mesenteric glands, and goat No. 2 showed the disease in a very early stage. In both cases the bacilli were found in the diseased tissues, and were isolated on special media (*vide* table, p. 126).

Satisfactory results with Vaccine No. 6 having been obtained, a further batch—Vaccine No. 7—was prepared as follows: We used our strain of Johne's bacillus that had become acclimatized to media containing no other acid-fast bacilli or extracts of such bacilli. It was grown for about four months on broth containing

1 per cent. peptone, ½ per cent. sodium chloride, and 4 per cent. by volume of glycerine. The cultures were shaken and the large masses of growth allowed to settle. The fluid containing the smaller particles was heated to 65° C. for one hour in a water-bath, and was used without previous filtration. This vaccine was tested on a number of adult bovines. Some were tested at the Brown Institution and others on farms by veterinary surgeons to whom we sent the vaccine. The results are summarized in the table, and we regret that we have been unable in most cases to obtain a post-mortem examination.

In the table the animals are numbered 4 to 25 inclusive, and it is unnecessary to describe each in detail, although the following points may be noted: No. 5, which failed to react to the vaccine, was a very advanced case of Johne's disease. It showed extensive lesions in the ileo-cæcal valve, in the lower part of the ileum, and in the cæcum. The upper part of the small intestine and the large intestine were less affected. An enormous number of bacilli were present in the mucous and submucous coats of the ileum (see Plates III. and IV.). The abdominal lymphatic glands were also extensively involved, and showed a large number of bacilli. In the opinion of the writers there is no doubt that this case failed to react owing to the very advanced stage of the disease. Cases Nos. 4, 6, and 9, on post-mortem examination, also showed extensive lesions of Johne's disease, but not so marked as in case No. 5. In each case the condition was most evident in the lower part of the ileum and in the ileo-cæcal valve. The abdominal lymphatic glands were only moderately involved. In all three cases a considerable number of bacilli were present in the ileum and a fair number in other parts of the intestine and in the

abdominal glands. Case No. 6 also showed tuberculosis of the bronchial glands. Cases Nos. 12 to 25 inclusive were all on the same farm, and were tested by ourselves. The animals were in a bleak, open position, and all were tested on a cold day. It is well known that when tuberculin is used under such conditions the temperature of a tubercular animal may not rise to the same extent that it would under more normal circumstances, and one would expect the same rule to hold good in animals affected with Johne's disease. If this is so, then the temperatures recorded in animals Nos. 12, 13, 23, and 25 all indicate good reactions, and in case No. 15 a fair reaction. It may be noted, too, that the temperature of each animal was subnormal before the vaccine was inoculated, and we were only able to take it at the fourth, sixth, and tenth hours following the inoculation of the vaccine. Case No. 22 showed definite clinical signs of tuberculosis, and gave no reaction with the vaccine.

More recently we have tested Vaccine No. 7 on five sheep experimentally inoculated. No. 1 was inoculated by the mouth, Nos. 2 and 3 intravenously, and Nos. 4 and 5 intraperitoneally. Ten weeks after inoculation the animals were tested with the vaccine, this being injected subcutaneously. The temperatures were taken hourly after the third hour, up till the eleventh hour following the inoculations, and they were taken again at the twentieth and twenty-fourth hours.

No. 1	gave a maximum	of	105·0° F. in 8 hours.
No. 2	,,	,,	105·2° F. in 6 hours.
No. 3	,,	,,	107·1° F. in 6 hours.
No. 4	,,	,,	106·8° F. in 7 hours.
No. 5	,,	,,	107·8° F. in 6 hours.

It will be observed that in every case the maximum temperature was registered between the sixth and

eighth hours following the inoculation. None of the animals showed any evidence of diarrhœa.

On reviewing the tests carried out on cattle, sheep, and goats, we may at once exclude those with Vaccines Nos. 1 to 5. These vaccines were prepared from early cultures of Johne's bacillus, and were too weak to cause any definite reaction in the animals. The results obtained with Vaccines Nos. 6 and 7 show that a vaccine made from a good glycerine-broth culture of Johne's bacillus is as reliable a reagent for Johne's disease as tuberculin is for tuberculosis, and, indeed, it would have been surprising had it been otherwise.

In the case of Johne's disease, it is interesting to note that the maximum temperature was usually recorded before the tenth hour—that is, much earlier than when tubercular animals are tested with tuberculin. The situation of the disease in the intestine and lymphatic glands might possibly account for this early rise, but a more feasible explanation would appear to be found in the nature of the lesions. Nearly all tubercular lesions are surrounded by a fibrous capsule, or are encompassed by a large quantity of lymphocytes, while in Johne's disease the lesions are never encapsulated, and gradually blend with the normal tissue.

As regards the specificity, we may note that all our tubercular animals that were free from Johne's disease, including some that are not given in the chart, failed to react in the slightest degree to a vaccine prepared from cultures of Johne's bacillus; while in animals suffering from Johne's disease, whether tubercular or not, a marked reaction was obtained. Exceptions, however, are found, as in very advanced cases, which may fail to react, and we know that this is often the

case when testing advanced cases of tuberculosis with tuberculin. As some of our cases of advanced Johne's disease were suffering from concomitant tuberculosis, the question arises as to whether the presence of the latter disease did not possibly mask the reaction in a few cases.

As in most biological tests, specificity can only be obtained by an exact system of dosage, and it is for this reason that the use of avian tuberculin in Johne's disease has been proved to be practically worthless. We have seen that in Johne's disease undoubted reactions were obtained by Bang, Miessner, etc., by the use of an avian tuberculin, but the reactions were more often negative than positive; and there is no doubt that to attain a larger percentage of positive results the dose of this vaccine would have to be such that it would be beyond the limits of reliability, and tubercular or even sound animals might give a reaction.

It is probable that in the future modified vaccines will be prepared from cultures of Johne's bacillus, and that other methods of inoculation will be resorted to. In the case of tuberculins many different methods of preparation and administration have been published, and we have seen that Bang, Malm, Male, and, more recently, M'Fadyean, Sheather, and Edwards, have tested some of these modifications on animals suffering from pseudo-tuberculous enteritis. It appears to us, however, unlikely that there will be much improvement on the subcutaneous inoculation of a vaccine prepared from an ordinary glycerine-broth culture of Johne's bacillus, although the ophthalmic test with the same vaccine may be useful under certain circumstances.

At the present day nearly all cattle have to undergo

the tuberculin test before being transported from one country to another, and it is to be hoped that the agricultural authorities of this and other countries will soon enforce the same regulations as regards Johne's disease, since a reliable diagnostic vaccine for this disease can now be prepared.

CHAPTER VIII

THE AGGLUTINATION REACTION AND THE COMPLEMENT FIXATION TEST

The Agglutination Test. — The presence of agglutinins in the serum of bovines suffering from Johne's disease can be demonstrated in the ordinary way (C. C. Twort); but, as in the case of tuberculosis, a positive reaction cannot be obtained with a highly diluted serum. Animals experimentally inoculated also produce agglutinins; not only bovines which subsequently contract the disease, but also such animals as rabbits and fowls, to which, up to the present time, the disease has never been transmitted. Needless to say, the sera of experimental animals should always be tested before the first injection is made, as the normal serum may possibly agglutinate. Although we have not found any normal rabbits give positive reactions, we have had to discard one or two fowls on this account. In considering the dilutions of the sera, they refer in all cases to a complete reaction, a partial agglutination often being obtained with a much higher dilution.

Technique.—This is extremely simple, and the same as that used for testing a suspected tubercular serum, with the following difference:

To obtain the best results in the last-mentioned cases it is usual to employ a homogeneous broth-

culture of the tubercle bacillus as recommended by Arloing. Owing to the slow growth of Johne's bacillus in the substance of a fluid medium, even though it contains a certain amount of an alcoholic extract of *B. phlei*, one is obliged to have recourse to an emulsion of the bacilli grown on solid media. As will be remembered, Koch and Romberg used an emulsion made from a growth on solid medium in their tubercle agglutination reactions. To obtain an emulsion of Johne's bacillus, the growth is mixed with saline, placed in a stout bottle containing glass beads, and shaken in an ordinary electric shaker. To obtain a good homogeneous emulsion, the usual precautions must be taken. The culture should be young, and after shaking should be filtered or centrifuged, to free the emulsion from large clumps of bacilli. Fresh unheated serum should be used, and it is best to perform the test as soon as possible after the withdrawal of the blood. This precaution does not refer to fowl serum, which agglutinates as well after heating at 56° C. as before. In this case, too, the serum may be kept in an ice-chest for months without any apparent diminution in activity. As in testing a tubercular serum, the mixture must be incubated for some time—two to four hours. If the microscopical method of examination is used, two hours is usually sufficient, and this method in our opinion is far more reliable than the macroscopic test. A control with the fresh unheated serum of a normal animal of the same species as that from which the suspected serum was obtained must, of course, be made. It is well also to have a second control, containing no serum, as spontaneous agglutination of the emulsion is liable to take place, especially if living bacilli are used.

The sera of seven naturally infected cattle, six of

which are numbered 1, 2, 3, 4, 5, 6 in the table on p. 126, were tested at the Brown Institution, and all gave positive reactions in a dilution of from 1 in 10 to 1 in 15; but it must be remembered that the serum of normal cows will usually agglutinate an emulsion of tubercule bacilli in a dilution of 1 in 5, or even 1 in 10 (Descos).

After testing the sera of five normal calves, two normal sheep, and two normal goats, which were all negative, the animals were inoculated with a pure culture of Johne's bacillus (see pp. 150 and 151). Six months later samples of blood were again taken, when it was found that the five calves gave positive results in a dilution of 1 in 5, whilst the remaining animals showed no trace of an agglutinin.

Amongst the ordinary laboratory animals the fowl appears to be by far the most suitable for the production of these antibodies; but even in this class of animal there is considerable variation as regards the amount of agglutinin obtained. In some cases the serum may agglutinate after a single subcutaneous inoculation in dilutions of 1 in 20 to 1 in 40, whilst in others repeated inoculations fail to produce anything like such a strong serum.

As might be expected, the experiments demonstrated that the agglutination reaction is not specific within the acid-fast group of bacilli. The serum of a tubercular animal will agglutinate an emulsion of Johne's bacillus, and *vice versa;* the dilution of the serum is, however, usually higher with its homologous bacillus.

The Complement Fixation Test.—This reaction can also be obtained in the same category of animals as mentioned under the agglutination test; but it is a more difficult and elaborate test than the latter, and

can hardly be performed to give any degree of accuracy except by those habitually carrying it out.

The technique to be followed is similar to that used in performing the reaction with a suspected tubercular serum. Special care should be taken to titrate accurately the complement before each series of bloods is tested. At the Brown Institution a simple emulsion of bacilli was used as antigen, but it is possible that a better antigen would be obtained if an extract made from diseased bowel were added to the bacillary emulsion, as recommended by Hammer for tuberculosis. On the other hand, it is worthy of note that Miessner and Kohlstock were unable to obtain any satisfactory results when they used as antigen an antiformin extract, etc., of diseased gut which they called "enteritidin."

The tests which are detailed below were carried out on seven naturally infected bovines at the Brown Institution, the animals being the same as those used for the agglutination tests (Nos. 1 to 6; see table, p. 126). Of these animals, Nos. 1, 4, 5, 6, and 7 gave positive reactions, No. 3 a doubtful reaction, and No. 2 a negative. A few of the animals were tested several times, and on every occasion gave the reaction with more or less the same intensity. It may be mentioned here that in most of the animals the disease was in an advanced stage. In five experimentally inoculated calves (Nos. 1*a*, 2*a*, 3*a*, 4*a*, 5*a*, Chapters VII. and IX.), mentioned also under the agglutination tests, which had received an emulsion of Johne's bacillus six months previously, the reactions were negative, except with No. 5*a*, which gave a slight positive result. At the time the blood was taken, these animals were really in the incubation period of the disease, as they showed no diarrhœa or any other symptoms; but, on

post-mortem examination, in most cases the gut was thickened, and pure cultures of acid-fast bacilli were recovered from the intestines of four of the cases.

In rabbits experimentally inoculated no difficulty was found in demonstrating the presence of a complement fixing antibody, but in the case of fowls all attempts proved unsuccessful. Unfortunately the complement fixation tests, like the agglutination reactions, proved to be specific within the acid-fast group of bacilli only in a very limited degree.

Since avian tuberculin has been used as a diagnostic reagent in this disease by O. Bang, Male, Miessner and Trapp, Malm, and others, it was thought advisable to carry out a number of comparative tests with the immunity reactions described above. These experiments, however, gave no indication that Johne's bacillus is more closely allied to the avian tubercle bacillus than to the human and bovine types. From the results obtained, it appears highly probable that neither the agglutination nor the complement fixation reaction will prove of much value as a practical diagnostic method in Johne's disease. As in tuberculosis, so, too, in Johne's disease; the inoculation of a diagnostic vaccine has been shown to be a far more delicate test, and, what is perhaps of still greater importance, it is more specific, and at the same time easier to perform.

CHAPTER IX

THE PATHOGENICITY OF JOHNE'S BACILLUS: INOCULATION EXPERIMENTS WITH INFECTED MATERIAL —INOCULATION EXPERIMENTS WITH PURE CULTURES OF JOHNE'S BACILLUS—THE PATHOGENICITY OF THE BACILLUS FOR SMALL ANIMALS

Inoculation Experiments with Infected Material.—As no cultures of Johne's bacillus were available at the time, all the early experiments regarding the pathogenicity of the bacillus were performed with infective material that was obtained at post-mortem examination.

The first to carry out systematic experiments in this direction were Johne and Frothingham, who sought to prove the tubercular nature of the condition by inoculating subcutaneously two guinea-pigs with small portions of the submucous tissue of the cæcum of a cow affected with the disease. In neither case was there any reaction at the site of inoculation; but the animals became considerably thinner, so that in spite of the absence of any local affection, they considered that the animals were suffering from a generalized tuberculosis. About five weeks after the inoculation, however, they were astonished to find that the condition of the animals had improved, and at the end of eight weeks both guinea-pigs were again in a perfectly sound condition. As a similar state of affairs may

sometimes occur with the avian tubercle bacillus, these authors concluded that they were dealing here with this micro-organism. It is now known that this was not so, and in the light of further experiments it is possible that the transitory illness of these animals was due to contaminating intestinal micro-organisms in the inoculated mucous membrane, and not to any toxic effect of Johne's bacillus.

In 1904, Markus inoculated subcutaneously six guinea-pigs and two rabbits, and fed two rabbits and three hens with infective material. The animals, all of which had increased in weight, were killed within periods of from 150 to 300 days, and were found post mortem to be quite normal. Stuurman, by feeding a rabbit with material from the intestine of an affected cow, produced abscesses in the intestines, and from these cultivated in pure growth an acid-fast bacillus which was afterwards proved to be the avian type of tubercle bacillus. B. Bang experimented with the diseased intestines and glands that he obtained from fourteen cows by inoculating a number of small animals. The material obtained from two of these cows, which came from tuberculous herds, produced typical tuberculosis in guinea-pigs, rabbits, and calves. Bang, however, produced Johne's disease in three calves by feeding them with large quantities (from 1 to 3 pounds) of the mucous membrane of a cow which was killed while suffering from the disease.

Miessner and Trapp, at the Agricultural Institute at Bromberg, made a number of animal inoculations with material from eight cases of naturally acquired Johne's disease. One of the experimental calves on post-mortem examination showed well-marked lesions of Johne's disease, but no signs of tuberculosis. The following experiments were made with material from

a cow which was suffering from Johne's disease, but was free from tuberculosis: The milk was centrifuged, and the sediment inoculated into six guinea-pigs and one calf, with negative results in six months. The urine was centrifuged, and the sediment inoculated into six guinea-pigs, which also gave negative results. The mucous membrane of the intestine was also inoculated into a number of animals. These are tabulated below, and show negative results with all the animals.

Six guinea-pigs subcutaneously, negative in four to five months.
Six guinea-pigs intra-abdominally, negative in four to five months.
Three rabbits subcutaneously, negative in four to five months.
Three rabbits intra-abdominally, one died in nine days (peritonitis), and the remainder were negative in five months.
Six pigeons subcutaneously, negative after five months.
One calf intravenously, negative after six months.
One calf *per os*, negative after six months.
Three hens *per os*, negative after four months.

A large series of inoculations was also carried out with the lymphatic glands attached to the mesentery. These are tabulated below, and in all cases show negative results.

Six guinea-pigs subcutaneously, negative after five months.
Six guinea-pigs intra-abdominally, negative after two to six months.
Three rabbits subcutaneously, negative after four months.
Three rabbits intra-abdominally, negative after three and a half months.
Two hens *per os*, negative after three and a half months.
One goat intravenously, negative after eight months.
One dog intravenously, negative after three and a half months.
One calf intravenously, negative in three months.
One calf intravenously, died in two days.

Negative results were also obtained with the spleen, udder, and lymph glands of the pleura, as shown below.

Spleen inoculated into four guinea-pigs subcutaneously, negative in four months.

Udder inoculated into two guinea-pigs intravenously, one negative in two months, one died in two days.

Lymph glands of pleura inoculated subcutaneously into two guinea-pigs, negative in two months.

Although in the naturally infected cow the bacilli were particularly numerous in the cæcum, it is interesting to note that all the inoculations, even in the calves and in the goat, gave negative results.

These authors, however, made similar experiments with seven other cows which showed lesions of Johne's disease; five of these were also tubercular. In some cases the small animals inoculated with infective material from those cows, which were tubercular, contracted tuberculosis; but, although a very large number of animals (including a sheep) were inoculated by various channels with infected intestine, Miessner and Trapp were unable to reproduce the disease in any species with the exception of three calves. Of these calves one was inoculated intravenously with mesenteric gland, and the remaining two with the intestinal mucous membrane of a cow suffering from Johne's disease. In these cases the disease was demonstrated on post-mortem examination. In each case the intestine was thickened, and films from the thickened portions showed numerous acid-fast bacilli. With the intestinal mucosa of one of these calves two more calves were fed and another inoculated subcutaneously. All three animals reacted to avian tuberculin five or six months later (see p. 114).

Working in 1907, M'Fadyean failed to produce any lesions in rabbits and guinea-pigs by inoculating the animals with infected intestine, and many other authors (Liénaux, Matthis, Fréger) have made similar unsuccessful attempts to infect the small laboratory animals. In other cases tuberculosis has resulted,

due, no doubt, to coincident tuberculosis in the cow from which the infected material was obtained.

Inoculation Experiments with Pure Cultures of the Bacillus.—When in 1910 the present writers obtained pure cultures of Johne's bacillus on a special medium (see Chapter VI.), a series of animal experiments was started. Care was taken to subculture the growths for several generations, in order that cultures might be obtained free from the diseased tissue that was placed on the original tubes. The strain used was the first that we isolated, and was obtained from the gland of the naturally infected case No. 2 (see p. 71). In the first instance we inoculated a cow and five calves, all of which were obtained from a herd of cattle that had been under the observation of a veterinary surgeon for three years, during which period no case of Johne's disease or any similar condition had occurred. One calf died seventeen days after the inoculation, and need not be considered further. Of the remaining cases, each is described below individually and in detail. Bovines Nos. 1, 2, 4, and 5 are also referred to on p. 122.

Bovine No. 1.—A shorthorn cow about eight years old and six weeks pregnant was tested in October, 1910, with ordinary tuberculin, and was found to be free from tuberculosis. On November 19, 1910, the animal was inoculated with a pure culture of Johne's bacillus. The growth was eight weeks old, and the third subculture distant from the original culture from the mesenteric gland. The growth was made into an emulsion with sterile 0·8 per cent. sodium chloride, and the whole given by the mouth to the cow, which had had no food for twelve hours. No immediate symptoms followed. From the time of feeding with the bacilli the temperature of the animal was taken twice daily, and it remained within normal limits, the averages for the

morning and evening over a long period being 101·2° F. and 102·2° F. respectively. Repeated examination of the fæces failed to reveal any acid-fast micro-organism resembling Johne's bacillus. On June 5, 1911, the cow calved. Parturition was normal and the calf quite healthy.

On October 23, 1911, she was killed, and a post-mortem examination made. The carcass showed marked emaciation, with practically complete absence of subcutaneous and intra-abdominal fat. The bronchial glands showed advanced tubercular lesions, while the pleura and the thoracic surface of the diaphragm showed a few recent tubercular patches. A few tubercular nodules were also present in the lungs. Some of the intestinal lymphatic glands also showed tubercular lesions of recent origin. All the remaining organs and tissues of the body, with the exception of the intestines, appeared normal. When the intestines were opened, a considerable thickening of the mucous membrane was observed. This was most marked in the vicinity of the ileo-cæcal valve, the terminal portion of the ileum, and the first part of the large intestine. No ulceration or caseation of the mucosa was present. The general thickening and corrugation of the mucosa presented the characteristic appearance of Johne's disease, and films made from beneath the mucous membrane showed a few small acid-fast bacilli. From the tubercular bronchial gland we obtained a pure culture of a bovine strain of tubercle bacillus; but all the cultures taken from the intestinal mucosa on to the special media remained sterile. It is unfortunate that the specific bacillus was not isolated from this case. Nevertheless, from the naked-eye appearance, and still more from the histological examination of the intestine, there is no doubt that this animal had con-

tracted Johne's disease as a result of the experiment, and it is to be presumed that the coincident tuberculosis was an accidental infection contracted by the animal while in the stables.

Bovine No. 2.—Heifer calf. Previous to inoculation, the temperature of this calf was taken twice daily for twelve days, and was found to be normal for an animal of its age. The fæces were also examined several times for acid-fast bacilli, with negative results. On November 10, 1910, when sixteen days old, the calf was inoculated with a culture of Johne's bacillus. The growth was three weeks old and the third subculture distant from the primary culture from the diseased gland. The growth was washed off with sterile 0·8 per cent. sodium chloride, and an opalescent emulsion obtained. This was inoculated into a vein of the left ear, but no immediate symptoms followed. The fæces were frequently examined for the presence of acid-fast bacilli, and from December 15, 1910, to January 17, 1911, films occasionally showed some thick acid-fast bacilli, which, however, did not resemble Johne's bacillus. These were probably some saprophytic "dung bacilli," and from the latter date no acid-fast micro-organisms were found.

On October 24, 1911, the animal was killed. On post-mortem examination, the carcass showed no emaciation, and no pathological lesions were found in any of the organs or tissues of the body with the exception of the bronchial lymphatic glands. These were slightly enlarged, and showed several caseating centres in which tubercle bacilli were demonstrated. On microscopical examination, the intestinal lymphatic glands failed to reveal any acid-fast bacilli. Cultures were made in the usual manner from one of the tubercular bronchial glands and from beneath the intestinal

mucosa. Those from the gland grew tubercle bacilli; the rest remained sterile. The fact that this animal accidentally contracted tuberculosis, while it resisted the inoculation of the culture of Johne's bacillus, demonstrates, with other experiments, a comparatively low virulence for Johne's bacillus.

Bovine No. 3.—This was a red and white male calf which was received at the Institution when three days old. Taken for about three weeks, the temperature remained normal for a young animal, and repeated examination of the fæces failed to reveal any acid-fast bacilli. On December 1, 1910, the calf was inoculated intraperitoneally with $\frac{1}{2}$ c.c. of a thick emulsion of Johne's bacillus, the growth being the second subculture distant from the primary culture from the gland. No immediate symptoms followed, and the temperature remained normal. Daily examination of the fæces, however, from December 29 to January 11, showed an increasing number of short, thick, acid-fast bacilli with rounded ends. Though these did not resemble Johne's bacilli as found in pure culture or in lesions, we decided to kill the calf and examine the intestine. This was done on January 11, 1911, and the post-mortem showed no pathological lesions. A few acid-fast bacilli were found in the mucous membrane and in sections of the bowel, but they appeared to be in the crypts of the membrane, and were probably not Johne's bacilli. We consider the result of this experiment negative, the calf being killed before the disease had had time to develop.

Bovine No. 4.—This was a brown male calf, and when received at the Institution (January 7, 1911) was two days old. The fæces showed no acid-fast bacilli, and the temperature remained normal up to February 21, when the calf was inoculated into the peri-

toneal cavity with about 5 milligrammes of a moist growth of Johne's bacillus. This growth was the fourth subculture distant from the primary culture. From the time of inoculation onwards the animal showed no definite symptoms, no acid-fast bacilli could be detected in the fæces, and, with the exception of the periods when various vaccine tests were made, the temperature remained normal. On October 16 the calf was killed. The animal was in good condition, and no lesions either of tuberculosis or of Johne's disease could be found. Films made from beneath the intestinal mucosa showed no acid-fast bacilli, and cultures from this situation remained sterile, so that the result was entirely negative.

Bovine No. 5 (*Calf No.* 5).—This was a red and white male calf, and was received at the Institution January 7, 1911. Repeated examination of the fæces failed to reveal any acid-fast bacilli, and the temperature remained normal. On February 21 the calf was inoculated intravenously with about 7 milligrammes of a moist culture of Johne's bacillus. The culture used was the fourth subculture distant from the primary growth. No immediate symptoms followed, and there was no rise of temperature. The condition of the calf remained good during March and the beginning of April, but towards the end of the month it became thin and weak. These symptoms increased up to May 11, when the calf was not able to rise without assistance, though the appetite was good and the temperature normal. In the early part of June the calf could again walk without assistance, but it remained very thin. There was no diarrhœa, nor could any acid-fast bacilli be found in the fæces at any time. On July 12, 1911, the animal was killed. The carcass showed extreme emaciation, and a small tuberculous

nodule was found in one of the bronchial glands; but there was no other evidence of tubercular disease in the body. The remaining organs and tissues, with the exception of the intestines, showed no pathological lesions, though there was very little fat present in any of the usual situations. The intestine, on examination, showed a moderate degree of thickening, especially in the ileum and cæcum near the ileo-cæcal valve. Films were made in the usual manner from beneath the mucous membrane, and after a prolonged examination several typical Johne's bacilli were found in those from the region of the ileo-cæcal valve. Cultures were made from a number of the glands and from the submucous tissue of the gut on to tubes of Dorset's egg medium. The tubes were capped with gutta-percha tissue and incubated at 39° C. After five days the small pieces of tissue were removed from the Dorset's egg medium and placed on to tubes of tubercle bacillus-egg medium and timothy-grass bacillus egg-medium. One tube of the latter medium, on which was placed material from the ileo-cæcal valve, showed definite microscopic evidence of growth in twenty-five days. This culture, the only one obtained, was easily sub-cultured on to fresh tubes of the special medium, but failed to grow on ordinary Dorset's egg medium. The acid-fast bacillus in the bronchial glands was isolated on Dorset's egg medium, and it possessed all the characteristics of a typical bovine tubercle bacillus. It is interesting to note that although this animal had contracted typical Johne's disease, yet, in spite of the tuberculosis, which might be expected to lower the resistance of the animal, the disease was in a very early stage. This experiment is further evidence of the slow progress of the disease.

The strain of Johne's bacillus isolated from this calf,

as has been mentioned, was obtained originally from the naturally infected case No. 2 described on p. 71. The culture obtained from the calf was again grown for about six months outside the animal body, and occasionally subcultured. A growth obtained in the third generation, which was about two and a half months old, was then inoculated into five calves. Calves Nos. 1*a* and 2*a* were inoculated intravenously, Nos. 3*a* and 4*a* intraperitoneally, and No. 5*a* subcutaneously.

After being tested with various vaccines, these animals were killed a little over six months from the date of inoculation, and, post mortem, all but one proved to be more or less affected (see table, p. 126).

Calf No. 1*a* showed typical lesions of Johne's disease in the intestines and mesenteric glands. The bacilli were most numerous in the tissues of the ileo-cæcal valve, but were also present beneath the mucous membrane of other parts of the gut, and in the glands. The animal showed no evidence of tuberculosis.

Calf No. 2*a* showed slight lesions of Johne's disease in the intestine and in the mesenteric glands, which were somewhat larger than normal. Several acid-fast bacilli were found in the glands, and a few beneath the mucous membrane near the ileo-cæcal valve. No tubercular lesions were found.

Calf No. 3*a* showed typical tubercular bronchial glands, but there was no evidence of Johne's disease in the intestine or mesenteric glands.

Calf No. 4*a* showed tubercular bronchial glands, which were caseous and in places calcareous. Johne's disease was present in a moderately advanced stage. The bacilli in the intestinal wall were most numerous near the ileo-cæcal valve, a situation in which we have usually found the lesions to be most pronounced. The

PLATE IX.

FIG. 1. FIG. 2. FIG. 3.

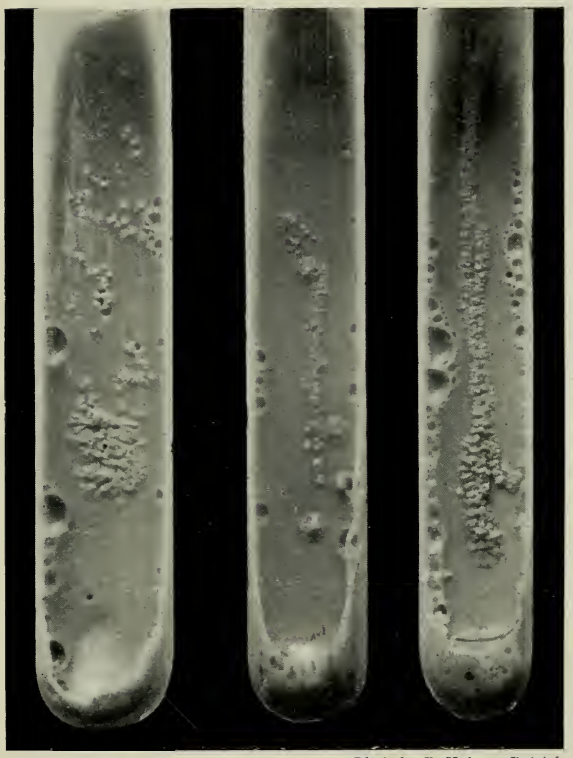

Photo by F. Holmes, Bristol.

CULTURES OF JOHNE'S BACILLUS.

FIG. 1.—Primary culture from experimental goat No. 1 on an egg medium made up with saline and containing 1 per cent. of dead *B. phlei* and 4 per cent. glycerine.

FIG. 2.—Primary culture from experimental goat No. 2 on same medium as Fig. 1.

FIG. 3.—Streak culture from experimental calf No. 5 on an egg medium made up with peptone-beef broth and containing 1 per cent. of dead *B. phlei* and 4 per cent. glycerine.

[*To face page* 150.

disease in the mesenteric glands was particularly well marked, due, no doubt, to the method of inoculation. Films from these glands showed a fair number of Johne's bacilli in each field.

Calf No. 5*a* was small and emaciated. On post-mortem examination it showed typical tubercular bronchial glands and early tuberculosis of the apex of the right lung. There was no definite thickening of the mucous membrane of the intestine, and the mesenteric glands were not much enlarged. Very few acid-fast bacilli were found beneath the intestinal mucosa, but several were present in films from one of the mesenteric glands.

All five calves showed some congestion of the mucous membrane of the intestines, with occasional hæmorrhages. Some of the glands also showed hæmorrhages, probably caused by the dose of vaccine which each animal had recently received (p. 128). These conditions were present only in a slight degree in calf No. 3*a*.

From all five cases cultures were made in the usual manner from the intestinal mucosa and abdominal lymphatic glands, and in all cases except No. 3*a* the bacillus was recovered in pure culture on a timothy-grass bacillus medium. Tubes of ordinary media inoculated at the same time from the intestine and glands all remained sterile. Thus, out of five calves, four had contracted the disease.

In July, 1911, we inoculated two young goats with living cultures of Johne's bacillus. The growth from one tube of medium was made into an emulsion with 10 c.c. of sterile 0·85 per cent. sodium chloride. Goat No. 1 received 3 c.c. of this emulsion intravenously; goat No. 2 was given 1 c.c. in the peritoneal cavity.

Eleven months later, after being tested with a vaccine

(p. 129), these two goats were killed. At this time one of the animals (goat No. 1) was thin, and the inoculation of the vaccine had caused severe diarrhœa; but in goat No. 2 there were no clinical manifestations of the disease. On post-mortem examination, goat No. 1 showed typical lesions of Johne's disease throughout the intestine and in the abdominal lymphatic glands. In films from both places the bacilli were present in fair numbers. Goat No. 2 showed the disease in a very early stage, and several bacilli only were found. From both these cases, however, following the usual procedure, we were able to isolate the bacilli on the special media (see Plate IX., Figs. 1 and 2).

From the positive results obtained in the two goats it is highly probable that these animals, like bovines, can contract the disease naturally, although, as has already been pointed out, Miessner and Trapp obtained negative results with goats that were fed with diseased gut from a cow.

Quite recently we have reproduced Johne's disease in sheep (*Veterinary Record*, April, 1913). Five animals were inoculated with cultures of Johne's bacillus isolated from a cow. Sheep No. 1 was inoculated by the mouth, Nos. 2 and 3 intravenously, and Nos. 4 and 5 intraperitoneally. Ten weeks after the inoculations the animals were tested with a diagnostic vaccine. Nos. 1 and 2 gave doubtful reactions, and Nos. 3, 4, and 5 good reactions (*vide* Chapter VII., p. 131).

Shortly after the vaccine tests, animals Nos. 3 and 5 were killed and post-mortem examinations made. Both showed definite lesions of Johne's disease, and no evidence of tuberculosis.

In the case of sheep No. 3, the intestines showed a moderate degree of thickening, most marked in the cæcum and ileo-cæcal valve, but only a few bacilli

were found. The mesenteric glands were considerably enlarged, but in these we were unable to demonstrate the presence of Johne's bacillus.

Sheep No. 5 showed no definite thickening of the intestine, but several acid-fast bacilli were found in the walls of the ileo-cæcal valve. The mesenteric glands were much enlarged and presented the typical appearance of Johne's disease, although only a few bacilli were present.

These experiments are interesting, since they prove that Johne's bacillus, isolated from a naturally infected bovine, is pathogenic for sheep, and is capable of producing a condition in these animals which is identical with that found in cattle, and we feel justified in assuming that the disease in naturally infected cattle and sheep is caused by one and the same micro-organism.

The Pathogenicity of the Bacillus for Small Animals.—Numerous attempts have been made to infect the smaller laboratory animals, such as rabbits, guinea-pigs, etc. As we have already mentioned, the earlier investigators, Johne and Frothingham, B. Bang, etc., used portions of the diseased bowel obtained from cattle suffering from this condition. The results, except when the inoculated material also contained tubercle bacilli, appear to have been entirely negative, indicating that these animals are endowed with a natural immunity against the disease in the same way as they are immune to the human lepra bacillus.

At the Brown Institution, working with pure cultures, the authors have inoculated pigeons, hens, rabbits, guinea-pigs, mice, and rats, subcutaneously and intraperitoneally, while several pigeons, hens, and rabbits were inoculated intravenously. These animals, together with some mice, rats, and rabbits, which were

fed with pure cultures of the bacillus, gave entirely negative results. In all cases single inoculations were made, and the animals were examined in periods varying from two months to two years after the inoculation. In one rabbit, inoculated intraperitoneally, a caseous nodule about the size of a large pea was found embedded in the intestinal wall; the nodule involved the peritoneal and muscular layers, and although a few acid-fast bacilli were found microscopically, they could not be recovered in culture, and were probably dead. The result must be considered negative.

More recently C. C. Twort and T. Craig have investigated the effect on rabbits and hens of massive doses of Johne's bacillus, not so much with the idea of reproducing the typical disease as with the intention of studying the toxic effects of the bacillus in comparison with other members of the acid-fast group, and the mechanism of the immunity of these small animals. The results detailed below refer to the experiments of these workers, and if one takes into account the size of the doses, they demonstrate the relatively low toxicity of Johne's bacillus.

The Intravenous Inoculation of Rabbits. — The inoculation of a single dose of 30 to 120 milligrammes of Johne's bacillus produces apparently no ill-effect on the animals, which eat well and show no loss of weight, while in a young animal the normal growth is unimpaired. There is no immediate or subsequent rise of temperature, or, at the most, a rise of 0·5° F. on the day following the injection. If a second injection is made three to five days later, there is again apparently no ill-effect, except, perhaps, some slight loss of appetite for a day or two, and the temperature remains practically constant. In a large number of animals inoculated with Johne's bacillus the rise of temperature

produced by the bacilli never exceeded 1° F., and in no case has a maximum of 104° F. been recorded. The normal temperature of a rabbit lies between 100° and 104° F., and, as is well known, slight causes produce considerable variation. Thus a rise of temperature that fails to reach 104·5° F. is not of much significance, except in those animals in which the temperature has been consistently low—*i.e.*, 100° to 102° F. before the inoculation.

Animals receiving a second inoculation usually remain quite healthy, but if a further dose is given five days later, some, after four, five, or six weeks gradually become emaciated, and ultimately die. If a second or third dose is given fifteen to thirty days after the first inoculation, a large proportion of the animals die within four weeks. Here, undoubtedly, one is dealing with animals that have already developed specific anticorps in their blood, etc., and the violent reactions sometimes observed shortly after the second inoculation are not dissimilar to ordinary anaphylactic shock, and the ultimate death of the animals is probably due to this cause.

Post-Mortem Examinations.—A single intravenous injection produces no macroscopic change in any of the organs, with the exception of the kidneys and lungs. Twenty-four hours after the inoculation the kidneys, on histological examination, are found to be congested; and this condition is more evident in animals killed on the third day. If the animals are killed after three or four weeks, no congestion or other pathological change can be detected. From the first day the lungs are usually somewhat congested, and may show hæmorrhagic points, although after a few weeks the normal condition is again found. Animals inoculated intravenously with two or more doses within five days,

and killed shortly afterwards, show much the same condition as is met with after a single injection. In those killed at a later period the organs usually show additional changes. The kidneys may, or may not, be congested, and sometimes they present slight evidence of cirrhosis. The lungs are usually normal in appearance, but they may be a little œdematous. The spleen is often somewhat enlarged, while the liver is more or less fatty throughout.

Animals that die after receiving a second intravenous dose fifteen to thirty days after the first injection, and those that succumb to repeated inoculations given at short intervals, can be considered together. In these cases post-mortem examination reveals an acute nephritis, and sometimes the presence of fluid in the peritoneal cavity. More rarely the pleural cavities also contain fluid. There is usually fluid in the pericardial sac, which is often distended to such a degree that one has no hesitation in attributing death to the presence of the fluid in this situation. The visceral layer of the pericardium is often somewhat rough, but, except for the presence of the fluid, there is not much evidence of pericarditis. The lungs, in most cases, are pneumonic. The liver may be congested, or pale and fatty, according to the length of time that the animal has survived. The spleen is generally normal, though it may be slightly enlarged. The bladder is usually distended with urine, whilst the lymphatic glands, such as those of the axilla, are often congested, sometimes intensely so. In none of these cases has any pathological change been found in the intestines, and nodular formations are absent from all the organs.

Microscopical Examination of the Organs.—The kidneys are congested from the first day, and already

show evidence of tubal desquamation, which appears to be most marked in the tubuli contorti. In the animals that receive a single injection, this inflammatory condition does not increase much in severity, and in a very short time the organ regains, more or less, its healthy state. When multiple doses are given, the changes described above are intensified, the kidneys show marked hæmorrhagic tubal nephritis, and in cases that have survived for any length of time an early interstitial nephritis may also be present. The nodular formations which follow the intravenous injection of most of the other acid-fast bacilli have not been observed with Johne's bacillus, and we have been unable to trace the passage of the bacilli through the kidney by means of stained sections. However, the bacilli are undoubtedly excreted by this organ, since they can occasionally be demonstrated in smears made from the stringy albuminous material usually present in the pelvis of the kidney. All attempts to obtain cultures from the urine have failed, although numerous specimens were taken from twenty-four hours to two months after the inoculation of the animal.

Congestion of the lungs is evident after twenty-four hours, and masses of acid-fast bacilli are found surrounded by a few epithelioid cells. The cells rapidly increase in number, and small foci appear in the interstices of the alveoli; these foci resemble on casual observation the early stage of a miliary tubercle. The majority of the bacilli are quickly phagocytosed, but those in clumps remain extracellular, and are surrounded *en masse* by epithelioid cells, lymphocytes, etc. The intracellular bacilli are often found in characteristic wreath-like formations, but they disappear completely on the tenth to the fifteenth day after the last inoculation. In animals receiving a single injection, the lung

gradually resume their normal healthy state, although in some cases the walls may remain somewhat thickened. In those that die as a result of multiple injections, the lungs are more or less completely solidified, and present a state of static pneumonia.

The spleen is not much affected except for a certain amount of congestion, although acid-fast bacilli are present from the first day, and persist for at least thirty days. From the beginning practically all are intracellular, but they invariably resist the action of decolorizing agents, and remain well formed or become somewhat granular. The Malpighian bodies are usually quite free from bacilli.

When present in the spleen, the bacilli are found also in the liver, and may be present from the first to the twentieth or thirtieth day after the last injection. They are phagocytosed by the interstitial cells (Kupffer's cells, sessile macrophages of Metchnikoff), but the true gland cells remain free from bacilli. Some investigators have maintained that the liver gland cells may, under certain circumstances, show phagocytic properties. C. C. Twort and Craig have obtained a remarkably clear picture of the phagocytic power of the interstitial cells, with total inactivity of the gland cells; the interstitial cells in many cases are crammed with bacilli. The liver soon becomes congested, and from the second to the third day, or even earlier, shows evidence of degeneration; the protoplasm becomes granular, whilst the nuclei remain well formed and stained. The condition is more marked in the hepatic than in the portal zone, as might be expected from the accompanying congestion of the organ. About the third day a lymphatic invasion commences around the portal vessels and bile-ducts; but in those animals that receive only a single injection it is not extensive, and

rapidly disappears, leaving a loose fibrous tissue. On the other hand, where the injections have been repeated, a large proportion of the parenchyma may be replaced by this loose fibrous tissue, and a general fatty condition of the remaining liver substance may supervene.

The intestines, when examined at different levels, show a complete absence of pathological changes, and, so far, it has been found impossible to demonstrate the presence of acid-fast bacilli in this situation.

In animals that receive several injections at long intervals, the axillary glands may be very congested, and may show small hæmorrhages, while acid-fast bacilli are often present.

As only a comparatively small number of bacilli seem to be excreted by the kidney, but, on the other hand, are rapidly absorbed by the liver, it was thought that many might pass through the bile-ducts into the intestines.

In order to prove this the following experiments were performed:

Two rabbits were inoculated intravenously with 30 milligrammes of the bacilli, one being killed twenty-four hours, and the other forty-eight hours, after the inoculation. A few drops of the urine and bile were placed on to separate tubes of the special medium necessary for the growth of Johne's bacillus, and incubated at 39° C., while the remainder of each of the fluids was centrifuged, and the deposit examined microscopically for bacilli. A careful examination of the deposits failed to reveal the presence of any micro-organisms, and the cultures appeared to be sterile at the end of three weeks. However, a week later several minute colonies were visible in the tube containing the bile from the rabbit killed forty-eight hours

after inoculation, and, on staining, bacilli with all the characters of Johne's bacillus were found.

A similar series of experiments, consisting in feeding rabbits with pure cultures of Johne's bacillus, were then carried out, but no bacilli could be found in the bile or urine, and cultures made from these fluids remained sterile.

The Intraperitoneal Inoculation of Rabbits.—The intraperitoneal inoculation of 100 to 200 milligrammes of Johne's bacillus is easily tolerated by rabbits, and seems to produce no ill-effect upon the general health; there is no rise of temperature or loss of appetite or weight, and no deaths directly due to the bacilli have been noted. If small quantities of the fluid contents of the peritoneal cavity are pipetted off a few hours after making the injection, and a microscopical examination made, there is evidence of leucocytosis, and the bacilli are found to be phagocytosed, only a few remaining free after twenty-four hours. Johne's bacillus, however, has a very great resisting power, and may be found well stained and well formed inside the phagocytes for several weeks.

In animals killed four weeks after inoculation all that can be seen on post-mortem examination is a very small amount of thick stringy pus in the peritoneal cavity. Rabbits inoculated into the peritoneal cavity and kept for two or three months are of special interest, as they are the first animals in which any evidence of nodular formation has been found. Usually these nodules are not numerous, and are limited to the abdominal cavity. They vary in size from a matchhead to a bean, and are indistinguishable from an ordinary caseous tubercular mass. The nodules occur on the under surface of the diaphragm, on the peritoneal covering of the liver and spleen, and in the

large omentum; often there is a fair-sized nodule on the cæcum. The last mentioned is frequently lobulated (like a gland), and may be simply attached to the intestine by a broad pedicle; it may, however, be embedded in the organ, involving the peritoneal coat.

The liver is often somewhat fatty, and on microscopical examination the condition of this organ and of the spleen is more or less identical with that found when the intravenous method of inoculation is used. The distribution of the bacilli in these organs is very similar; they are present twenty-four hours after the inoculation, and, as in the intravenous cases, may persist for a month. The caseous nodules referred to often contain enormous numbers of small acid-fast bacilli, presumably Johne's bacilli; but it has been found impossible to recover them in pure culture, due, no doubt, to their being dead. Sometimes the number of bacilli is small, as in the intestinal nodule of the rabbit inoculated intraperitoneally by the authors.

Acid-fast bacilli have not been demonstrated in any of the remaining organs, all of which appear to be normal.

The Subcutaneous Inoculation of Rabbits.—The subcutaneous inoculation of Johne's bacillus into rabbits produces a caseous abscess at the site of inoculation. These abscesses persist for a great length of time, but the most interesting feature is the resistance of the bacilli to destruction in this situation; they can be found in large numbers many months after the inoculation, and remain well stained and formed.

If dead bacilli are inoculated in place of living they show an equally marked resistance to destruction. Up to the present time not a single acid-fast bacillus has been found in any of the internal organs of these animals after subcutaneous inoculation.

Fowls inoculated intravenously with Johne's bacillus develop lesions more or less similar to those found in rabbits after intravenous inoculation, but only a very limited number of these animals have been tested.

A Comparison with Other Acid-fast Bacilli.—C. C. Twort and T. Craig have performed a considerable number of experiments on rabbits with other members of the acid-fast group, their object being to compare the toxicity of these bacilli with that of Johne's bacillus. The following bacilli were tested:

1. *Bacillus phlei* (Moeller).
2. Smegma bacillus (Moeller).
3. Marpmann's bacillus from urine.
4. Paratubercle bacillus (Binot).
5. Mist bacillus (Moeller).
6. Pseudoperlsucht (Moeller).
7. Nasenschleim bacillus (Karlinski).
8. Duval's "so-called" leprosy bacillus.
9. Tobler I.
10. Tobler II.
11. Grassberger's bacillus.
12. Fish tubercle bacillus (Dubard).
13. Grass bacillus (Moeller).

Nearly a hundred animals were inoculated with these bacilli, and a subsequent examination of the organs was made. In the majority of rabbits *B. phlei* was used; only two or three animals were inoculated with each of the remaining types of bacilli.

It is upon the following points that the chief differences appear to rest:

1. The general toxicity of the bacilli and the length of time the animals survive after the inoculation.

2. The power of the bacilli to cause a definite rise of temperature.

3. The excretion of the bacilli by the kidney, and the production of nodules in this organ.

4. The production of caseous nodules.

5. The cultivation of the bacilli from the urine of the inoculated animals.

The bacilli detailed above may be divided into two

groups, according to their toxicity. Nos. 1 to 7 may be considered as more or less toxic, and the remainder as comparatively non-toxic. The description given below applies entirely to the intravenous method of inoculation. With Nos. 1 to 7 there is always a definite rise of temperature to 105° or 106° F.; it reaches 105° F. twenty-four hours after inoculation, and rises another 0·5° or 1° F. on the following day. On the fourth or fifth day there is a fairly sudden fall, and at the end of the week the normal is again reached. Loss of appetite and consequent wasting are also present, the latter being in most cases very marked. The animals usually die within five to ten days, although they may temporarily recover, and not succumb until two or three months later, while in some cases recovery appears to be permanent.

From the second to the third day onwards the bacilli are excreted in large numbers by the kidneys, and may be recovered in pure culture from the urine twenty-four hours after inoculation. The kidneys are riddled with nodules after the fifth day, these nodules being formed entirely of lymphocytes, bacilli, and cells of the involved kidney substance. If the animal survives, the bacilli rapidly disappear, and the nodules become absorbed without any caseation. It is worthy of note that in this situation the bacilli attain a great length, and are easily decolorized after staining. Caseous nodules may be found in the lungs, liver, spleen, and peritoneum, and in some cases nodules formed of invading lymphocytes are present in the heart muscle and suprarenal glands. Of the six remaining bacilli, Duval's so-called leprosy bacillus is the only one that produced a temperature of 105° F., and this result has been obtained in two rabbits. Animals inoculated with these six varieties of bacilli

do not die, the bacilli being less toxic than Nos. 1 to 7. The only varieties that produce nodules in the kidney are Tobler I. and Grassberger's bacillus, but the nodules are very few in number. Cultures have been obtained from the urine with Nos. 9 to 11, whilst those made from the urine of animals injected with Nos. 8, 12, and 13 have all proved negative. In animals inoculated with the fish tubercle bacillus and with Duval's bacillus, caseous nodules have been found in the peritoneum, but attempts to trace the passage of the bacilli through the kidney by microscopical examination of sections of this organ have all given negative results.

All the bacilli under consideration are more or less non-toxic if inoculated into the peritoneal cavity or subcutaneously, and the same is true if they are killed by heating and injected intravenously. By the term "non-toxic" is meant that there is no rise of temperature, or loss of appetite or weight, and the animal does not die after the inoculation of 100 to 200 milligrammes of the bacilli. That some of these bacilli are, to a certain degree, pathogenic for animals if inoculated into the peritoneal cavity or subcutaneously, has frequently been demonstrated by many investigators. The lungs of some of the rabbits inoculated subcutaneously with *B. phlei* have consisted, to a large extent, of necrosed tissue, yet the animals, while alive, appeared to be quite healthy. In one case hardly one-third of the normal lung tissue remained.

On reviewing the comparative experiments, one is forced to assume that the difference between these acid-fast bacilli is one of degree only; but in dividing them into groups, toxic and non-toxic, it is clear, from what has already been said, that Johne's bacillus is one of the least toxic of the non-toxic group. When

inoculated intravenously, it is highly probable that all the varieties are excreted, to some extent at least, by the kidney. It is easy to understand the negative cultural results obtained with the fish tubercle bacillus and with Moeller's grass bacillus, both of which are probably rapidly destroyed in the animal body owing to the existing temperature; it seems, however, rather curious that it is impossible to obtain cultures of Johne's bacillus, since the temperature here does not come into play. It must be remembered that only a limited number of animals have been used in these experiments, so that a certain amount of reserve must be exercised in drawing conclusions from the results.

The Resisting Power of Johne's Bacillus to the Destructive Agents in the Animal Body.—While showing a high degree of resistance to decolorizing reagents, such as mineral acids and alcohol, Johne's bacillus is, at the same time, very difficult to destroy in the animal body. As we have seen, when inoculated into the peritoneal cavity of rabbits, the bacilli are rapidly phagocytosed; but they may be found a month later within the leucocytes, resisting well, after staining, decolorizing reagents, and being either normal in appearance, or, at the most, somewhat granular. *B. phlei* and the human tubercle bacillus are also rapidly phagocytosed, but they soon disappear almost entirely from the peritoneal fluid, and the same has been found to occur in the peritoneal cavity of mice. Caseous nodules produced by tubercle bacilli in the peritoneal cavity contain but few bacilli, while those caused by Johne's bacillus may be crowded with bacilli. In rabbits immunized by repeated subcutaneous inoculations of dead human tubercle bacilli, and subsequently inoculated into the peritoneal cavity with living bacilli of the same species, or with Johne's bacillus the

tubercle bacilli are found to disappear the more rapidly. This might be expected, but, on the other hand, in animals immunized with dead Johne's bacillus, and then inoculated with one of the two living bacilli as before, it is again the human tubercle bacillus that first disappears.

If, as is thought by some authors (Wolff-Eisner, etc.), the tuberculin reaction be due to the action of the specific lysin on the tubercle bacilli, or particles of them, then one would expect the rise of temperature in the tuberculin test to take place at an earlier hour than in animals suffering from Johne's disease, and treated with a diagnostic vaccine prepared from Johne's bacillus. The contrary, however, seems to take place, since the tuberculin reaction appears about the ninth hour to the eighteenth hour, whilst, as we have seen, the reaction in the case of Johne's disease usually takes place before the ninth hour. It is, however, possible that the comparatively early disappearance of tubercle bacilli is not due to lysis, but to the fact that they are more toxic than Johne's bacillus to the cells, which break down more quickly, and liberate the bacilli, these subsequently becoming disseminated throughout the animal body. It is also well known that in an encapsulated caseous nodule tubercle bacilli are usually not numerous, whilst we have seen that Johne's bacillus is often present in enormous numbers. The same appears, in a general way, to be true as regards the destruction of the bacilli in subcutaneous abscesses.

That (lysed ?) Johne bacilli are as toxic as the (lysed ?) tubercle bacilli is proved by the general disturbance caused in animals by the inoculation of a diagnostic vaccine. The same is shown by experiments performed on rabbits that have been immunized

by intravenous inoculation of Johne's bacillus, *B. phlei*, or the human tubercle bacillus. Five to ten milligrammes of any of these bacilli often produce rapid death of the animal if inoculated intravenously, while in many cases a previous high rise of temperature is noted. This rise of temperature is usually preceded by a well-marked fall, which occurs during the first hour or two following the injection. When the inoculation is made subcutaneously, the temperature often rises but little, and the experiment never terminates fatally. It requires no larger dose of dead Johne's bacillus to kill an animal immune to the human tubercle bacillus than it does of the latter to kill an animal immune to Johne's bacillus. The bacillary emulsions have not been accurately titrated to find the minimal fatal dose, and it is, of course, assumed that the dose would be smallest in those cases in which the homologous bacillus is used. Control animals, immunized with emulsified Dorset's egg medium to eliminate any effect of the egg albumin in these reactions, were negative, both as regards the production of a rise in temperature and the death of the animal.

If mice are inoculated into the peritoneal cavity with dead Johne's bacillus, *B. phlei*, or the human, avian, or bovine types of tubercle bacilli, the most prominent feature is the more rapid disappearance of the four last-mentioned varieties as compared with Johne's bacillus. It is about a week or more after the injection that the difference is most noticeable. At the same time animals inoculated with Johne's bacillus do not appear to die so frequently as when inoculated with the other bacilli.

Experiments *in vitro* on the toxicity of the bacilli to guinea-pig's leucocytes are also interesting. The leucocytes can be obtained in the usual way by the intraperitoneal injection of Mellin's Food,[1] or similar

substance. They are collected, centrifuged, and washed, and six drops are added to one drop of a homogeneous emulsion of the bacillus to be tested, together with two drops of a normal guinea-pig's serum, and the mixtures are incubated at 37° C. In every case, if the emulsion be not too thick, phagocytosis is complete in about twenty-four hours. The comparative non-toxicity of Johne's bacillus for the leucocytes is shown by the fact that the remaining tubes containing human, bovine, or avian tubercle bacilli, or *B. phlei*, soon become contaminated, showing death of the leucocytes ; while the tube containing Johne's bacillus remains sterile for a longer time, and the leucocytes appear normal. On the other hand, these are partially degenerated in the tube containing *B. phlei*, and are completely so in those containing tubercle bacilli.

From what has been said above it is clear that Johne's bacillus has a low degree of toxicity, especially for such animals as rabbits. As we have seen, in cattle that suffer naturally from Johne's disease toxic symptoms are very little in evidence, and the temperature remains constant throughout, showing a lack of any general disturbance of the animal economy, whilst the absence of local necrosis seems to indicate that the bacillus has no very harmful influence on the neighbouring cells. The rise of temperature sometimes met with in very advanced cases is probably due to a secondary infection with intestinal micro-organisms the general resistance of the animal being low owing to malnutrition. In some cases the number of bacilli found on post-mortem examination is small, and from this certain authors have assumed that the symptoms accompanying the later stages of the disease are directly caused by highly toxic substances secreted by the bacilli. The view, however, generally held is

that the toxicity of Johne's bacillus is not great, and that a diseased animal is but little affected until the large masses of bacilli, and the pressing of new tissue on the glands, lead to derangement of food absorption, malnutrition, and diarrhœa ; all the observations and experiments of the authors tend to confirm this view. In an intestinal disease the number of factors influencing the general health must be considerable, and, as we have already seen, an exactly opposite condition of affairs may exist—*i.e.*, the presence of a large number of bacilli with a comparatively robust state of health of the animal.

We have already seen that certain investigators reproduced the disease in calves, but the doses of infected material used were often very large, and sometimes repeated. The experiments of the authors with pure cultures of the bacillus gave the same results. To reproduce the disease in a susceptible animal, it was necessary to inoculate much larger doses of Johne's bacillus than would have been necessary to produce tuberculosis with the tubercle bacillus.

But, even when the typical disease is present, it must be remembered that the bacilli and lesions are confined to the intestine and glands, and the question arises as to whether there are not, in the case of Johne's bacillus, special factors, besides the production of toxins by the bacillus and the active resistance of the host, which help to determine its pathogenicity, and in the case of susceptible animals, its distribution. It *may* be that in the body, as in artificial culture media, special foodstuffs are necessary for its vitality, and that these exist in sufficient quantity only in the walls of the intestine and in the mesenteric glands of certain ruminants. It is to be presumed that such foodstuffs cannot exist in large quantities, even in the intestine,

otherwise Johne's bacillus would grow on a broth prepared from such tissues, or on pieces of fresh gland removed aseptically, and this we know is not the case. What is far more probable is that the necessary food exists in very small quantity, but is constantly replenished as it is used by the bacillus. As we have pointed out (Chapter VI.), we have evidence that the substance contained in the body of *B. phlei*, which stimulates the growth of Johne's bacillus in artificial cultures, is an organic acid; and it is probable that it is some such acid—possibly in combination—existing in the intestinal wall, which stimulates the growth of the bacillus in this situation. Such an acid might be a product of metabolism of the neighbouring glands, or a product of digestion passing through the intestinal walls, or, indeed, it might be produced from such substances as grass by the action of certain intestinal bacteria, and be absorbed by the intestine, thus reaching the vicinity of the bacilli. However this may be, the facts remain that the disease is confined to the intestine and mesenteric glands of certain ruminants, and that a large dose of infective material is necessary to reproduce the disease experimentally. From this it seems probable that under good hygienic conditions a healthy bovine has but little chance of infection if a single dose of infective material is taken with the food. Repeated ingestion of infected food, especially by animals poor in condition, is probably essential for infection in the majority of cases.

Considering now the case of rodents and birds, we have already seen that these do not naturally contract the disease, and that very large doses, and in some cases repeated doses, are necessary to produce pathological changes; but even in this case the bacilli rapidly die, and cannot be recovered in pure cultures.

In rabbits the temperature is never high, and the absence of other signs of intoxication is a marked feature. The natural immunity of these animals to Johne's bacillus is not sufficient to safeguard them against a fatal termination of the experiment, and we have seen that the saprophytic *B. phlei* may cause rapid death of the animal, although the latter is endowed with an immunity against this bacillus. This may be appreciated more easily by a brief consideration of what takes place in animals inoculated with *B. phlei.* Although *B. phlei* is a saprophytic microorganism, when inoculated intravenously into rabbits it causes a rise of temperature within the first twenty-four hours. This is presumably due to a toxin secreted by the bacillus, the animal possibly producing antibodies to the toxin, which may neutralize it completely by the fourth or fifth day after the injection, at which time, as we have seen, the temperature falls more or less to the normal.

An intraperitoneal or subcutaneous inoculation produces no rise of temperature. In these cases it is probable that the toxin is absorbed by the liver or subcutaneous tissue, and thus does not reach the heat-regulating centres of the brain. That the crisis is not caused by the death of the bacilli inside the animal body, or by the excretion of the bacilli, is proved by following closely the condition of the kidneys. It is true that cultures may be obtained from the urine twenty-four hours after inoculation, but on microscopical examination of the organs the number of bacilli present is very small, and in some cases they cannot be found even after a prolonged examination.

On the other hand, it is not until the third or fourth day that the kidney becomes invaded by a large number

of bacilli, and the bacilli increase in number up to the eighth or ninth day, long after the temperature has fallen. However, it must be remembered that at this stage, although the bacilli are numerous, they are surrounded by a dense wall of small round cells, and it is significant that the appearance of these cells coincides with the fall of the temperature. A detailed discussion of these facts is hardly within the scope of this book; it only remains to be borne in mind that such a condition of affairs does not exist when Johne's bacillus is inoculated. The ultimate death of the animal appears to be due to the pathological condition of the kidney.

If now we consider Johne's bacillus, we find a condition similar to that obtained when using killed *B. phlei.* The absence of a rise of temperature is probably due either to the rapid death of the micro-organism, or to the lack of formation of any free toxin; we are inclined to favour the latter hypothesis in view of the similar condition found in naturally infected bovines. The survival of rabbits is undoubtedly due to the maintenance of the normal condition of the kidneys, through which the bacilli are slowly filtered, and owing to the death of the bacilli, or to their inability to grow in this situation, the kidneys remain more or less in an uninjured state.

Thus it appears that although Johne's bacillus is pathogenic for certain ruminants, it is, nevertheless, one of the least toxic of the acid-fast group of micro-organisms, and we have seen that the majority of animals possess a natural resistance against the bacillus. We believe, too, from the distribution of the bacillus in naturally affected animals, and from the experiments on the cultivation of the bacillus, that the question of a special food-supply may play an impor-

tant part in determining the pathogenicity of the bacillus and its distribution in the body of a naturally affected animal.

Since this book was written C. C. Twort has succeeded in reproducing in a rabbit a pathological condition similar to that found in cattle and sheep in the early stages of Johne's disease. This he has done by the intravenous inoculation of a culture of Johne's bacillus which has become acclimatized to grow on ordinary glycerine-beef broth. On post-mortem examination the mucous membrane in the vicinity of the ileo-cæcal valve was thickened, and the abdominal lymphatic glands were large and œdematous; bacilli were found in both situations, and there was no evidence of caseation. The disease in the intestine did not extend more than a foot from the ileo-cæcal valve. It may be noted that the animal was inoculated with two doses of Johne's bacillus.

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